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# BOTANICAL SCIENCES

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VOL. 24, SEC. C. JUNE, 1946 NUMBER 3

# INVESTIGATIONS ON RUBBER-BEARING PLANTS

## III. DEVELOPMENT OF NORMAL AND ABORTING SEEDS IN *ASCLEPIAS SYRIACA* L.[1]

BY RAYMOND J. MOORE[2]

### Abstract

A low percentage (2 to 3%) of the flowers of *Asclepias syriaca* L. produce mature seed pods. Many ovaries begin to enlarge but abort before reaching maturity. Histological examination of these pods shows that ovule abnormalities accompany pod collapse. Ovules in free-nucleate endosperm stages show a growth of the integument that is excessive for ovules at this stage of embryo sac development. Older aborting ovules contained a zone of collapsed cells completely enclosing the endosperm.

Seed collapse of the former type at least appears similar to cases attributed to a competition for food between endosperm and the adjacent maternal tissue of the ovule, termed "somatoplastic sterility" by Brink and Cooper.

### Introduction

Early in 1942 the Division of Botany and Plant Pathology of the Department of Agriculture, in co-operation with the National Research Council of Canada, initiated a comprehensive series of investigations on plants that were potential supplementary sources of natural rubber. This Division was responsible for study of the cytogenetics, physiology, and agronomy of these plants as a means of determining whether they could be economically grown in Canada.

One of the species that received major attention was *Asclepias syriaca* L., common milkweed. Wartime interest in this species also developed as a result of the use of milkweed floss as a substitute for kapok in life-preserving equipment. Early observation showed that very few milkweed flowers produced mature pods. This fact was of great importance from the standpoint of utilization of floss and also from that of rubber production since large quantities of seed were needed to plant commercial acreages. The investigation reported below was undertaken as an attempt to explain the failure of many flowers to produce pods.

[1] *Manuscript received January 17, 1946.*

*Contribution No. 843 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Earlier papers in the series "Investigations on rubber-bearing plants" were published as follows: I. The propagation of Taraxacum kok-saghyz Rod. by means of leaf cuttings. Botan. Gaz. 107 : 260-267. 1945. II. Carbohydrates in the roots of Taraxacum kok-saghyz Rod. Can. J. Research, C, 24 : 47-53. 1946.*

[2] *Agricultural Assistant.*

## Field Observations

Observations of the growth of seed pods revealed that only 2 to 3% of the flowers produced mature fruits. A high percentage of the flowers fell 10 to 12 days after flowering. These flowers showed no enlargement of ovaries or of flower pedicel. Ovaries that survived this period began growth, but all did not reach maturity. Death of these ovaries occurred at widely varying stages. Ovaries showing only slight enlargement as well as pods as long as 6 cm. dried and shrivelled up. These however did not fall from the plant but remained firmly attached to the peduncle by the thickened pedicel. The percentage of enlarged ovaries that failed varied widely; all or none or any intermediate fraction of the developing pods in one umbel might abort at any stage of growth and at periods up to two and one-half to three weeks after flowering.

## Materials and Methods

Material for histological study was obtained from plants growing in experimental plots in the Dominion Arboretum and Botanic Garden, Ottawa, Canada. The plants were grown from seed collected at several stations in and near Ottawa. The soils of the various plots used were clay–loam and loam in nature.

All plantings flowered freely during July of their second year and fixations were made during July and August of 1944 and 1945.

Ovules of normally developing pods and of enlarged but apparently aborting ovaries were fixed in Randolph's Nawashin, embedded, and prepared for microscopic study in the usual manner. Safranin and fast green, with the occasional addition of gentian violet, and Heidenhain's iron alum haematoxylin were the stains used.

## Literature Review

Frye (7) has described the development of the ovule and macrogametophyte and followed the development of the fertilized embryo sac as far as the initiation of the cellular condition of the endosperm. A single integument and a deeply sunken nucellus constitute the maternal tissues of the ovule. A hypodermal cell of the nucellus functions directly as the macrospore mother cell and from this cell a row of four macrospores is formed by the meiotic divisions. Usually, but not always, it is the innermost of these that develops into an eight-nucleate, seven-celled embryo sac of the Normal-Type (11). The mature embryo sac therefore contains a flask-shaped egg cell, two linear synergids, a large vacuolate two-nucleate primary endosperm cell, and three antipodal cells. The polar nuclei may or may not fuse before fertilization occurs. In the present study fusion before fertilization was more commonly seen. The nucellus is early absorbed by the embryo sac and none is present at maturity.

Double fertilization occurs and is immediately followed by division of the endosperm nucleus. Endosperm development is of the free-nucleate type, the early mitoses producing free nuclei that lie in a peripheral layer of cyto-

plasm about the central vacuole of the endosperm cell. At the fourth series of mitoses, by which 16 nuclei are formed, wall formation begins and thereafter the endosperm is multicellular. At this time, the zygote, until now undivided, divides by transverse wall.

## Development of the Normal Ovule

The present study agrees with that of Frye as to the structure of the mature ovule and early postfertilization development. Up to the eight-nucleate endosperm stage there has not been a great increase in the size of either the ovule or embryo sac, these being $100\mu \times 150\mu$ and $30\mu \times 100\mu$ respectively. Nor has increase in the thickness of the integument become conspicuous, for during the free-nucleate stage of the endosperm development this remains about six cells thick lateral to the embryo sac (Fig. 3). A strand of elongate cells along the funicular side of the ovule passes to the level of the antipodal end of the embryo sac. No differentiated vascular tissue is present in this strand and it is not connected directly with the embryo sac.

Rapid growth of the integument occurs after the endosperm has become septate and the zygote begins to divide. Early proembryos (Fig. 9) were found in pods 15 to 20 mm. in length. The integument (Fig. 8) was by now quite thick and the tuft of floss well begun.

In Fig. 14, a 4.5 mm. seed of a 40 mm. pod, it will be noted that the endosperm is a tissue of considerable size, at the micropylar end of which lies a spherical embryo (Fig. 13) with a multicellular suspensor. The endosperm cells are generally large and vacuolate with no reserve food. The integument shows some differentiation into layers. A true vascular strand, five to six cells thick, extends beneath the epidermis along the funicular side to the level of the chalazal end of the endosperm and there ends with no definitely differentiated zone connecting it with the embryo sac. Within the vascular strand or in regions where no vascular tissue is present immediately beneath the epidermis is a region about seven cells thick of compact, smaller, roughly isodiametric parenchyma. The cells lying within this compact zone are larger and more loosely packed and constitute a region about 7 to 14 cells thick. Adjacent to the endosperm is an area of two to three layers of empty, collapsed cells. Continuous with the micropylar end of the embryo sac and extending through the mid region of the integument to the region from which the floss arises is a channel of elongate usually empty cells.

Seed of 110 mm. pod presents a similar, though more advanced picture. At this time the embryo is greatly enlarged and shows a well formed radicle and cotyledons. It is embedded in endosperm the cells of which are thin-walled and contain dense protoplasts, apparently well supplied with reserve food material. This is essentially the condition found in the mature seed. The integument is differentiated into the same regions as previously described, epidermis, vascular strand, compact and loose parenchyma (less definitely distinguishable here), and an inner zone of collapsed cells extending in a central channel to the floss region.

The seed coat of a mature seed consists of two distinct layers.

1. Peripheral corky layer, one cell thick.

2. Thicker multicellular layer of remnants of walls of collapsed cells. In this layer traces of the vascular strand can be recognized along the funicular side.

The corky layer originates from the epidermis of the integument and the thicker layer from the collapsed cells of the integument from which the protoplasts have been absorbed by the endosperm.

## Floss Development

A tuft of silky hairs (the milkweed floss of commerce) is found on one end of the flat oval-shaped mature seed.

The origin of these hairs has been described by Frye (7) and by Netolitzky (9). They arise at the placental end of the ovule between the base of the funiculus and the micropyle when the ovule, as seen in longitudinal section, has become elongate and roughly rectangular. Each hair results from the elongation of an epidermal cell of the integument (Fig. 15). The epidermal cells become richly protoplasmic and elongate rapidly. The nucleus moves out to the mid region of the cell but no division follows and the mature hair is a uninucleate single cell.

Floss development is initiated slightly earlier, relative to the embryo sac stage, in abortive than in normal ovules. In the former, cell elongation begins at the eight-nucleate endosperm stage (Fig. 4) and, in normal ovules, when the ovule has become approximately the size of the abortive ovule containing free-nucleate endosperm. Normal ovules of this size contain cellular endosperm and early proembryos.

## Development of Ovules in Aborting Pods

Pods that were slow growing or appeared shrunken or yellowish were fixed for histological study. These ranged in size from those scarcely larger than the mature ovary to pods 2 cm. in length.

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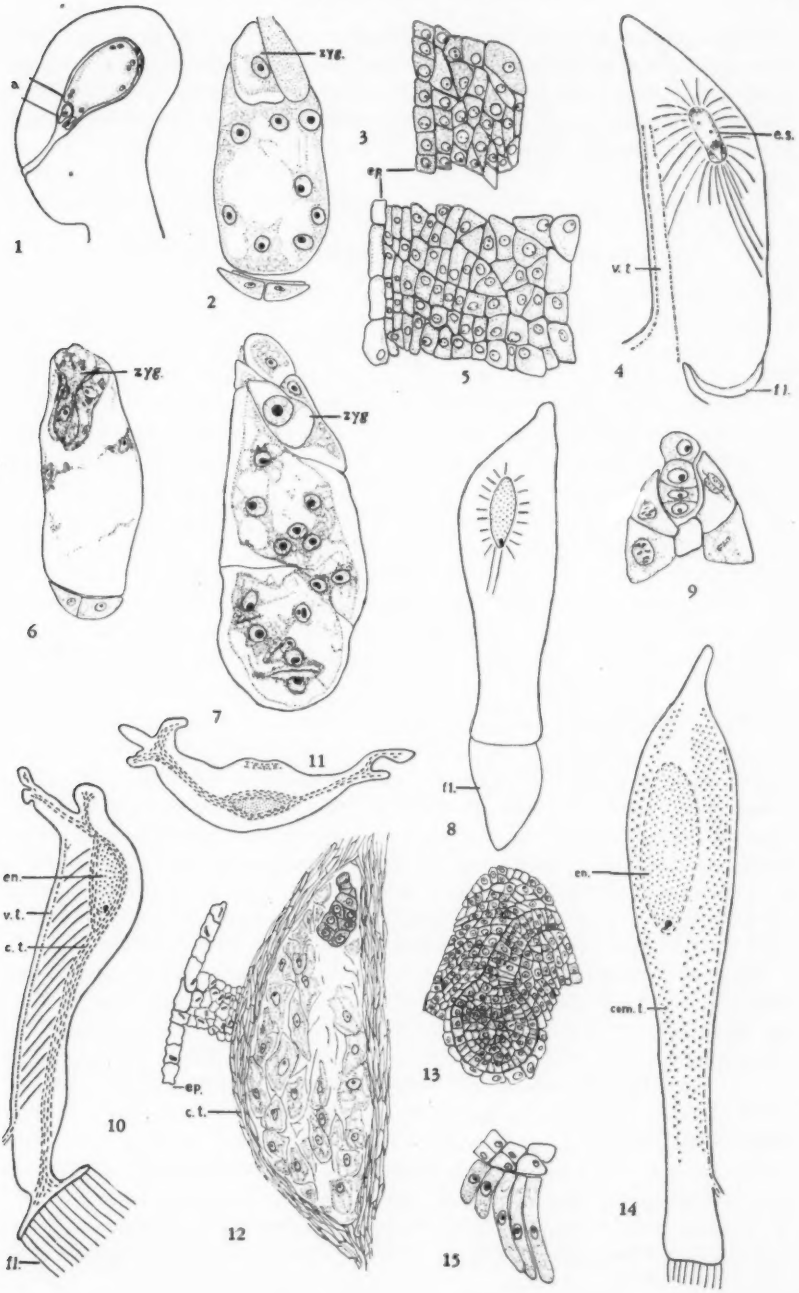
EXPLANATION OF FIGURES

FIG. 1. *Normal ovule, eight-nucleate endosperm stage.* ×*185.* FIG. 2. *Normal embryo sac, eight-free-nucleate endosperm.* ×*450.* FIG. 3. *Section of integument of Fig. 1, in region "a".* ×*490.* FIG. 4. *Hyperplastic ovule, eight-nucleate endosperm stage.* ×*90.* FIG. 5. *Section of integument of Fig. 4, corresponding to Fig. 3.* ×*490.* FIG. 6. *Abnormal embryo sac at two-nucleate endosperm stage, from hyperplastic ovule.* ×*450.* FIG. 7. *Abnormal partially septate embryo sac at 16-nucleate stage, from hyperplastic ovule.* ×*450.* FIG. 8. *Normal ovule at four-celled proembryo stage.* ×*50.* FIG. 9. *Normal four-celled proembryo, with adjoining endosperm.* ×*410.* FIG. 10. *Aborting ovule with multicellular endosperm and embryo and zone of collapsed cells.* ×*50.* FIG. 11. *Transverse section of ovule in Fig. 10 at level of endosperm.* ×*50.* FIG. 12. *Endosperm, embryo, collapsed tissue, and portion of integument of ovule in Fig. 10.* ×*200.* FIG. 13. *Embryo and adjoining endosperm of ovule in Fig. 14.* ×*200.* FIG. 14. *Normal ovule from 40 mm. pod.* ×*20.* FIG. 15. *Epidermal cells of integument elongating to form floss.* ×*325.*

LEGEND: *com.t.—compact tissue; c.t.—collapsed tissue; en.—endosperm; ep.—epidermis of integument; e.s.—embryo sac; fl.—floss; v.t.—vascular tissue; zyg.—zygote.*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The ovules of these pods contained embryo sacs in both the free-nucleate and cellular endosperm stage. All ovules with free-nucleate endosperm and undivided zygote were distinguished from normally developing ovules at the corresponding stage of embryo sac development by the excessive growth of the integument that had occurred prematurely (Fig. 16).

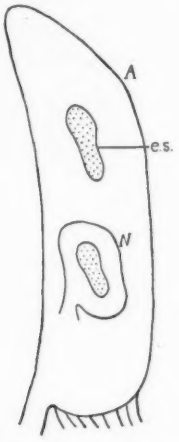


FIG. 16. *Outlines of normal ovule (N) and of aborting ovule (A), both at eight-nucleate endosperm stage. Both ovules drawn to same scale to show relative sizes.*

The integument was composed of thin-walled undifferentiated cells. In the zone about the embryo sac the cells, roughly rectangular in section with the longer axis parallel to the margin of the embryo sac, were arranged in regular compact rows appearing as if radiating from the periphery of the endosperm. Distal to this compact zone the cells were larger, roughly isodiametric, and not oriented in any special manner. Generally the cytoplasm of the cells of the former region stained more densely and the nuclei were larger than those of the looser parenchymatous region. The embryo sacs of most such ovules appeared abnormal and obviously degenerate.

Aborting ovules containing multicellular endosperm and embryos were characterized by a zone of empty collapsed integument cells that enclosed the embryo sac on all sides (Figs. 10, 11) and extended through the middle of the integument into the wings. The embryo sacs were in an obvious state of breakdown, the endosperm cells being shrunken and separated from one another. The embryo was as yet in an apparently more normal condition than the endosperm.

Ovules of a single pod were at similar stages of integument and embryo sac development. Hyperplastic ovules and unfertilized ovules occasionally were found in the same pod but as yet no such occurrence of hyperplastic and normal ovules has been seen.

Abnormality of the free-nucleate endosperm was apparent in the distorted shape of the nuclei and in the shreddy and coagulated appearance of the cytoplasm (Fig. 6). No doubt this appearance was exaggerated by fixation but probably an unusual physiologic condition of the protoplast was the basis of the abnormal reaction to the fixing fluid. Embryo sacs of normal ovules were not plasmolysed in this manner. Occasionally a partially-septate condition of the endosperm cell at the 8- to 16-nucleate stage was seen (Fig. 7).

## Description of Specific Cases of Aborting Ovules

Abortion may set in immediately after fertilization. Such was the case of an ovary 2.5 mm. in length. The ovules, 150$\mu$ × 200$\mu$ in size, contained embryo sacs with undivided zygotes and endosperm in the one- to two- or four-free-nucleate stage. The integument was 11 to 12 cell layers thick lateral to the sac, about twice the thickness of that of corresponding normal ovules. The endosperm was obviously in a state of disintegration, the cytoplasm being badly distorted by fixation and shrunken into coarse strands and blotchy masses. Some of the nuclei were faint in outline, as though the nuclear membrane had broken down. Frequently the zygote was disintegrating and appeared as a dense shrunken mass of cytoplasm in the midst of which a darker spot, probably the remnant of the nucleus could be seen. The synergid cells were represented by elongate masses of darkly staining cytoplasm. An embryo sac with two-nucleate endosperm is shown in Fig. 6. As in comparable normal ovules, no breakdown of the antipodal cells had yet occurred in such ovules. Occasionally a small deposit of apparently extracellular debris was seen between the antipodal cells and the adjoining integument cells. The debris did not appear to be the remnant of collapsed cells.

Aborting pods containing ovules in later stages of development also were characterized by the conspicuous premature thickening of the integument and an abnormal condition of the endosperm. The 4- to 16-nucleate endosperm stage was found in ovules the dimensions of which fell within the range of 150 to 200$\mu$ × 400 to 650$\mu$. The embryo sacs measured 30 to 50$\mu$ × 60 to 100$\mu$. The integument was 11 to 19 cell layers thick lateral to the embryo sac and 20 to 28 cells deep at the antipodal end. No true vascular tissue was present in ovules aborting in these early stages but a strand four to six cells thick of undifferentiated, elongate cells extended along the funicular side to the level of the antipodal end of the embryo sac. Conspicuous deposits of reserve food in the integument were lacking. With respect to the vascular development, collapsing seeds were similar to early normal ovules.

The extent to which disintegration of the endosperm had progressed varied somewhat in different ovules. Often the endosperm was not visibly abnormal but from the condition of the integument it could be safely judged that the ovules were destined to die before maturity. Disorganization of the endosperm was to be noticed in the distorted and plasmolysed condition of the tissue similar to that described for earlier stages.

Occasionally a partially septate condition of the endosperm at the 16-nucleate stage was noted (Fig. 7). In normal ovules the endosperm becomes septate at this time whereas in aborting ovules the 16-nucleate endosperm cell was divided into three to four irregular cells. The nuclei were embedded in coarse blotchy masses of cytoplasm and between some pairs there was an appearance of a spindle arrested in the process of cell-plate formation. The cells of the integument bordering on these ovules were not collapsed. Up to the 16-nucleate endosperm stage the zygote was undivided. No food reserve was noticeable in the endosperm.

Larger collapsing pods contained ovules with multicellular endosperm and early embryos. As previously mentioned, these ovules were characterized by a conspicuous zone of empty collapsed cells enveloping the embryo sac, as seen in Fig. 10 in longitudinal section and in Fig. 11 in transverse section. This condition was seen in ovules of failing pods 0.3 to 2 cm. in length.

Collapsed cells constituted the layers of the integument immediately adjacent to the embryo sac. Distal to this zone lay the yet normal cells of the integument and epidermis. The width of the collapsed zone and of the region of normal parenchymatous integument varied in different ovules, according to the extent to which seed collapse had progressed. In the extreme, no normal integument remained, all the cells having gone into the formation of the dead zone enclosing the embryo sac. The protoplasts of this central zone of integument are normally less dense than those of more peripheral cells in advanced ovules but the existence of such a zone of collapsed tissue at such an early stage of embryo sac development is definitely abnormal. A well-developed vascular strand, containing differentiated vascular cells passed down the funicular side of the ovule to the level of the antipodal end of the embryo sac. The roughly isodiametric cells of the integument were oriented in well-defined rows, passing obliquely from the vascular bundle to the periphery of the collapsed zone.

As seen in Fig. 12 the endosperm was in an advanced state of breakdown, the individual cells being shrunken and distorted and separated from one another. The multicellular proembryo, which consists of a few-celled suspensor and globular embryo proper, appeared to be in a relatively healthier condition than the endosperm. To judge by appearance, the endosperm failure seemed to precede that of the embryo, a condition frequently noted in studies on collapsing seeds.

## Unfertilized Ovules

Unfertilized ovules are readily distinguishable from aborting seeds. In the former no evidence of activity is visible. The egg cell and primary endosperm nucleus remain undivided and no development of the integument either by cell multiplication or cell enlargement takes place. Breakdown of the ovule finally follows, being first noticeable in the starved appearance of the integument cells. As degeneration proceeds, the integument cells, first those in the regions remote from the funiculus, empty and the walls shrink and collapse. Collapse of the embryo sac also shortly occurs.

## Discussion

It has frequently been observed in orchard fruits that a considerable percentage of flowers fail to produce mature fruits. This unsuccessful group is made up of ovaries that apparently never began development, and, as well, many ovaries that had enlarged but died before reaching maturity.

Studies of fruit development of orchard fruits have shown that the drop of immature fruits occurs in a number of distinct waves. Examination of the fruits has revealed that abnormality and early degeneration of the macrogametophyte and lack of fertilization are responsible for part of the drop. However, in a high percentage, definite evidence of fertilization and early embryo growth was reported.

A considerable number of studies on fruit collapse in pear, apple, plum, sour cherry, sweet cherry, and grape have been reviewed by Brink and Cooper (3, 5). Visible evidence of the cause of the seed failure was not found in some cases, whereas in others an excessive development of one of the maternal tissues of the ovule was observed.

In extensive investigations of seed abortion in alfalfa, Brink and Cooper (1, 2, 4, 6) found that such an excessive growth of the maternal tissue adjacent to the embryo sac occurred in failing ovules. These ovules showed unusual growth of the integuments and, apparently following this, collapse of the endosperm. Last of all, the embryo breaks down.

Such hyperplasia of the maternal tissue adjacent to the macrogametophyte has been termed "somatoplastic sterility" by Brink and Cooper (1). Comparable phenomena were found to accompany seed collapse after certain interspecific crosses of *Nicotiana*, *Petunia*, and *Lycopersicon* (5), of *Datura* (10), and of *Nicotiana* (8).

Brink and Cooper considered that a competition for the available food supply takes place between the endosperm and the adjacent ovular tissue. The ability to utilize the nourishment is proportional to the rate of nuclear division. In ovules that aborted, the rate of endosperm nuclear division was observed to be slower than that of ovules that reached maturity. The slower growth rate left more food available to the maternal tissue, which was thus stimulated to excessive development.

As a result of the competition for food the physiologically non-vigorous endosperm was starved and death of the embryo followed. Since the breakdown of the embryo appeared always to follow that of the endosperm, the endosperm failure was held to be the primary cause of seed collapse.

The lack of vigour of the endosperm was attributed to its unfavourable genetic constitution, received in the combination of incompatible genomes following interspecific pollinations or to a homozygous condition of probably recessive deleterious factors in the case of intraspecific pollination in alfalfa (5). In this species abortion was observed to be five times more frequent after self- than after cross-pollination.

The premature enlargement of the integument and the accompanying embryo sac degeneration seen in the smaller aborting ovules of *Asclepias syriaca* closely parallel the conditions associated with seed collapse in alfalfa. Here abortion of ovules in the free-nucleate endosperm stage was clearly distinguished by a multiplication of cell layers associated only with later stages of embryogeny in normal ovules. Although in most cases the endosperm showed evidence of disintegration, there were cases where no visible abnormality of the embryo sac could be noted. Nevertheless, in such ovules, the integument was definitely hyperplastic, indicating that abortion would doubtless soon follow. From such observations it may be concluded that the excessively rapid integument growth is the earlier morphological abnormality and that probably the unhealthy condition of the embryo sac is a result of the former activity.

Only in aborting ovules containing advanced multicellular endosperm and embryo was the zone of collapsed tissue about the embryo sac seen. Whether collapse of these seeds also can be attributed to tissue competition is less certain. The integument of successful seeds at a comparable stage is also extensively developed. Whether, in ovules destined to abort, development was initiated earlier than is normal cannot be decided from the material available. If so, it may be that the endosperm in spite of a slower growth rate was sufficiently vigorous to reach an advanced stage. The food available may have been so much diverted to the premature integument growth that, to survive, the endosperm was forced to absorb the contents of adjacent cells. Possibly also the collapse of the inner region of the integument represents the ordinary process of seed maturation occurring prematurely with respect to endosperm and embryo growth.

It is, however, also possible that an interruption of the food supply to potentially normal seeds has occurred and in an attempt to survive the endosperm has drawn upon the adjacent integument, causing the collapse of these cells. . No morphological evidence of the cause of such an interruption could be detected. A general starved appearance of integument, funiculus, and placenta prevailed but neither block in the vascular tissue nor chalazal abnormality was apparent.

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The writer wishes to express his sincere gratitude to Dr. Margaret Landes and Dr. H. A. Senn for their advice during the course of the study and in the preparation of the manuscript.

## References

1. Brink, R. A. and Cooper, D. C. Somatoplastic sterility in *Medicago sativa*. Science, 90 : 545-546. 1939.
2. Brink, R. A. and Cooper, D. C. Double fertilization and development of the seed in angiosperms. Botan. Gaz. 102 : 1-25. 1940.
3. Brink, R. A. and Cooper, D. C. Incomplete seed failure as a result of somatoplastic sterility. Genetics, 26 : 487-505. 1941.

4. COOPER, D. C. and BRINK, R. A. Partial self-incompatibility and the collapse of fertile ovules as factors affecting seed formation in alfalfa. J. Agr. Research, 60 : 453-472 1940.
5. COOPER, D. C. and BRINK, R. A. Somatoplastic sterility as a cause of seed failure after interspecific hybridization. Genetics, 25 : 593-617. 1940.
6. COOPER, D. C., BRINK, R. A., and ALBRECHT, H. R. Embryo mortality in relation to seed formation in alfalfa (*Medicago sativa*). Am. J. Botany, 24 : 203-213. 1937.
7. FRYE, T. C. A morphological study of certain Asclepiadaceae. Botan. Gaz. 34 : 389-413. 1902.
8. KOSTOFF, D. Ontogeny, genetics and cytology of *Nicotiana* hybrids. Genetica, 12 : 33-119. 1930.
9. NETOLITZKY, F. Anatomie der Angiospermen-Samen. *In* Handbuch der Pflanzenanatomie by K. Linsbauer. Bd. 10. Gebrüder Borntraeger, Berlin. 1926.
10. SATINA, S. and BLAKESLEE, A. F. Fertilization in the incompatible cross *Datura stramonium* × *D. metel*. Bull. Torrey Botan. Club, 62 : 301-312. 1935.
11. SCHNARF, K. Contemporary understanding of embryo-sac development among angiosperms. Botan. Rev. 2 : 565-585. 1936.

# INVESTIGATIONS ON RUBBER-BEARING PLANTS

## IV. CYTOGENETIC STUDIES IN *ASCLEPIAS* (TOURN.) L.[1]

By Raymond J. Moore[2]


### Abstract

The chromosome number of eight species and varieties of *Asclepias* (*A. syriaca*, *A. incarnata* and variety *pulchra* and forma *albiflora*, *A. Sullivantii*, *A. tuberosa*, *A. curassavica*, *A. speciosa*) was found to be $2n = 22$.

Various interspecific pollinations were attempted without success. A cross of *A. incarnata* with forma *albiflora* was successful.

Possible natural hybrids between *A. syriaca* and *A. speciosa* and between *A. incarnata* and var. *pulchra* and forma *albiflora* are described. Two unusual chimaeral plants of *A. incarnata* that produced several stems of forma *albiflora* are described.


### Introduction

In the course of recent wartime investigations of the possible economic uses of the genus *Asclepias*, chiefly the species *A. syriaca* L. and *A. incarnata* L., collections of these two and of several other species were assembled. As a preliminary to breeding work, the chromosome number of all the species and varieties was determined. Numerous attempts at intraspecific crosses of *A. syriaca* were made to work out a technique for artificial pollination of the curiously modified *Asclepias* flower. In addition, interspecific pollinations, involving all of the species available, were undertaken with a view to obtaining a hybrid that would surpass the native species in either latex or floss production.

### Materials and Methods

Seed was collected from native plants of *A. syriaca*, of *A. incarnata*, and of *A. Sullivantii*. Seed of other species was obtained from various Botanic Gardens. Plants were grown in plots at the Dominion Arboretum and Botanic Garden, Ottawa.

Herbarium specimens from these cultures are to be found in the Herbarium of the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa.

Chromosome counts were made from fresh smears of growing leaves and root tips fixed in Carnoy's mixture, made up as follows:

3 parts chloroform,
2 parts 95% ethanol,
1 part glacial acetic acid.

[1] *Manuscript received March 12, 1946.*

*Contribution No. 852 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Earlier papers in this series were published as follows: I. The propagation of Taraxacum kok-saghyz Rod. by means of leaf cuttings. Botan. Gaz. 107(2) : 260-267. 1945. II. Carbohydrates in the roots of Taraxacum kok-saghyz Rod. Can. J. Research, C, 24 : 47-53. 1946. III. Development of normal and aborting seeds in Asclepias syriaca L. Can. J. Research, C, 24 : 55-65. 1946.*

[2] *Agricultural Assistant.*

The tissue was then stained in 45% aceto-orcein, heated, and flattened on the slide.

For one species, *A. curassavica*, root tips fixed in Allen's Bouin fluid, embedded, sectioned, and stained with Gentian violet were studied as well.

## Cytological Observations

The diploid chromosome number of six species and two subspecific forms of one of these species was found to be 22. These counts, with previously reported determinations, are listed in Table I.

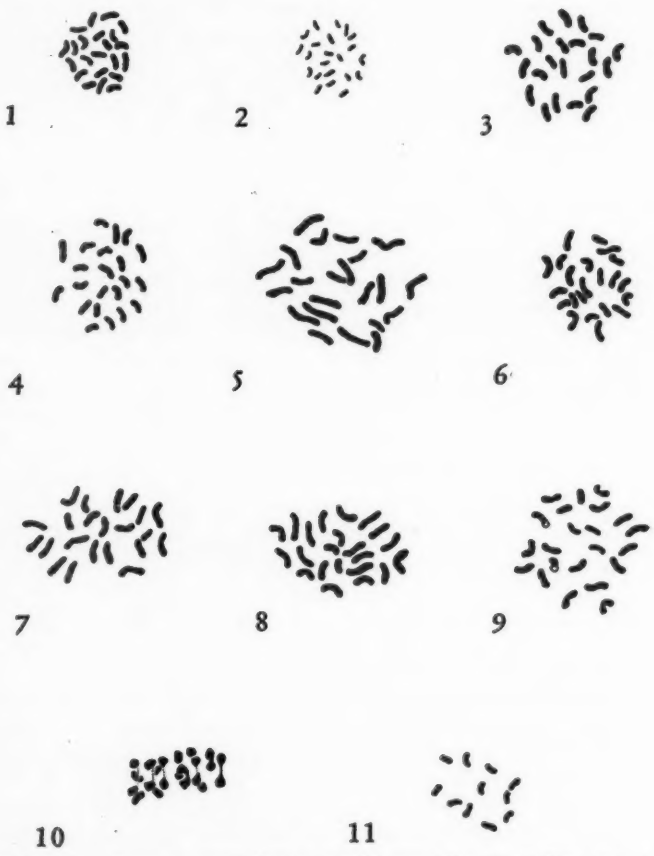
TABLE I

CHROMOSOME NUMBERS IN *Asclepias* (TOURN.) L.

| Species | *n* | 2*n* | Author |
|---|---|---|---|
| *curassavica* L. | | 22 | Moyer, 7 |
| *curassavica* L. | | 22 | Moore |
| *incarnata* L. | | 22 | Moyer, 7 |
| *incarnata* L. | | 22 | Moore |
| *incarnata* L. forma *albiflora* Heller | | 22 | Moore |
| *incarnata* L. var. *pulchra* (Ehr.) Pers. | | 22 | Moore |
| *latifolia* Raf. | | 22 | Moyer, 7 |
| *salicifolia* Lodd. | | 22 | Moyer, 7 |
| *speciosa* Torr. | | 22 | Moore |
| *Sullivantii* Engelm. | *ca.* 5 | | Frye, 5 |
| *Sullivantii* Engelm. | | 22 | Moore |
| *syriaca* L. | 10 | | Strasburger, 11 |
| *syriaca* | 12 | | Stevens, 10; Gager, 6; Finn, 2 |
| *syriaca* | | 22 | Eichhorn and Franquet, 1 |
| *syriaca* L. | | 24 | Moyer, 7 |
| *syriaca* L. | 11 | 22 | Moore |
| *tuberosa* L. | 5 | | Frye, 4 |
| *tuberosa* L. | | 22 | Moore |
| *tuberosa* L. var. *sulfurea* L. | | 22 | Moyer, 7 |
| *verticillata* | *ca.* 8 | | Frye, 5 |

The chromosomes varied in length, in the preparations used, from 1 to 2.5 $\mu$. It is not possible to recognize with certainty any of the homologous chromosomes although two or three pairs are somewhat longer than the others. Moyer (7) remarked that there was a noticeable difference in the morphology of the chromosomes of the different species. His figures show the chromosomes of *A. curassavica* to be smaller than those of five other species (see Table I). In the present investigation it was noted that the chromosomes of this species, in both root tips and leaf smears (Figs. 1, 2) were smaller than those of the other species examined. Differences between the chromosomes of the latter species were very slight. Some variation in morphology is very likely due to the physiological condition of the tissue and the rate at which mitoses have been taking place. For this reason it is felt that further comment on this variation is not justified.

In view of the disagreement (Table I) over the chromosome number in *A. syriaca* L. (*A. cornuti* Decaisne) special attention was paid to this species. Plants grown from seed collected from eight roadside stations about Ottawa, Ont. were examined but only the diploid number of 22 was found (Fig. 10). In Fig. 11 is shown the haploid number of 11 at II meiotic metaphase while in Fig. 10 are seen the 11 bivalents of I metaphase. Several additional counts of $2n = 22$ were made by Dr. Margaret Landes, Division of Botany and Plant Pathology, Department of Agriculture.




*All figures were drawn at a magnification of 2000 except Fig. 10,* $\times 1700$. FIG. 2 *shows somatic chromosomes from root-tips; Figs.* 1 *and* 3 *to* 9 *show the diploid chromosomes from leaf tissue.*

1. *A. curassavica* L. 2. *A. curassavica* L. 3. *A. incarnata* L. 4. *A. incarnata* forma *albiflora* Heller. 5. *A. incarnata* variety *pulchra* (Ehr.) Pers. 6. *A. speciosa* Torr. 7. *A. Sullivantii* Engelm. 8. *A. tuberosa* L. 9. *A. syriaca* L. 10. *A. syriaca* L. I *meiotic division,* 11 *bivalents.* 11. *A. syriaca* L. II *meiotic division,* $n = 11$.

## Hybridization

*Technique*

As is well known, the *Asclepias* flower is highly modified for insect pollination. The female parts, two separate ovaries, are almost entirely enclosed by a tube formed by the flattened filaments of the five stamens. Pollen is developed in flat club-shaped sacs (pollinia) of which there are two in each stamen. A thin tough band (retinaculum) fastens the pollinia in pairs, one member from each of two adjacent stamens. The upper portion of the styles of the two ovaries are united into a flat round stigma disk. Spaced at equal intervals around the margin of the lower surface of the disk are five stigmatic areas. Each stigmatic surface is thus located within a chamber, the outer walls of which are formed by portions of two adjacent flattened filaments, and is accessible to pollination only through a slit-like passage between the edges of the two filaments. By means of the retinacula, pollinia are readily entangled on the legs of insects gathering nectar and cross-pollination is effected by the insertion of one or more pollinia into the stigmatic chambers of the flower that the insect visits next.

In artificial pollinations, the pollinia were withdrawn from their pouches by means of a needle or fine pointed forceps and inserted into the chambers with an inward and upwards motion. Care was taken to cause as little injury as possible to the filament wings covering the entrance to the chamber. It was found after some preliminary attempts that no success was attained unless at least two adjacent chambers were pollinated with at least one pollinium each. From the arrangements of the stigmatic surfaces relative to the free styles, it appears that a passageway for pollen tubes to the same ovary will exist from four of the five possible pairs of adjacent stigmas. It was estimated that a pollinium of *A. syriaca* contains approximately 250 pollen grains. The greatest number of mature seeds obtained from a single pod was 243. It appears, therefore, that germination of a very high percentage of the pollen grains is required to fertilize all of the ovules present. Probably all of the grains in a sac do not germinate, or, if they do, cannot reach the stigmatic surface. It may be that pollen tubes from two stigmas are needed to fertilize a sufficient number of ovules to cause the ovary to remain attached to the stem.

Mature but unopened buds were used in artificial pollinations. The clusters were protected from subsequent insect pollination by paper bags. Usually only three to five flowers in an umbel were manipulated, the others were removed.

Following this technique a considerable number of intraspecific cross-pollinations of *A. syriaca* were successful.

The following interspecific hybridizations were attempted. The number of flowers crossed was relatively small. The approximate total number is indicated after each pollination. In all cases the female parent is named first. Pollinations in which some ovaries enlarged but aborted before maturity are indicated by an asterisk.

*A. syriaca* × *A. tuberosa* (50)
*A. syriaca* × *A. incarnata* (28) and *A. incarnata* f. *albiflora* (6)
*A. syriaca* × *A. speciosa* (10) and reciprocal (15)
*A. speciosa* × *A. tuberosa* (20)
*A. Sullivantii* × *A. syriaca* (15)
*A. Sullivantii* × *A. speciosa* (7)
*A. Sullivantii* × *A. tuberosa* (9)*
*A. incarnata* × *A. incarnata* v. *pulchra* (20) and reciprocal (30)
*A. incarnata* × *A. incarnata* f. *albiflora* (20) and reciprocal (12)
*A. incarnata* v. *pulchra* × *A. incarnata* f. *albiflora* (6) and reciprocal (20)
*A. curassavica* × *A. syriaca* (6)*
*A. curassavica* × *A. tuberosa* (9) and reciprocal (4)
*A. curassavica* × *A. incarnata* v. *pulchra* (4)
*A. curassavica* × *A. incarnata* f. *albiflora* (5)
*A. curassavica* × *A. speciosa* (4)* and reciprocal (6)*

Mature pods were obtained only in the cross *A. incarnata* × *A. incarnata* f. *albiflora*. It is felt that difficulties of manipulating the smaller flowers of *A. incarnata* were largely responsible for failure to obtain seed of the *A. incarnata* variety crosses.

After certain of the above cross-pollinations, as indicated, definite enlargement of the ovary was observed. The pod however did not mature and only small shrivelled seeds were found within. It is possible that fertilization occurred in these crosses but the seed aborted before maturity.

Seed gathered from *A. speciosa* plants in experimental plots in which *A. syriaca* also was growing produced plants that appeared to be hybrids between these species. Probably insect pollination was successful in effecting this cross. These possible hybrids showed intergradations in the leaf, flower, and stem characters of the two species.

Leaves of *A. syriaca* vary considerably in form and size—from almost round to linear-ovate and from 7 to 20 cm. in length—while those of *A. speciosa* are cordate-oval or oblong, generally smaller and covered with a silvery bloom, due to a fine pubescence. Typical *A. syriaca* plants reach 9 to 12 dm. in height, while those of *A. speciosa* are generally about 6 dm. The two species are separated most easily by their floral characters, especially the length of the hood. Hoods of *A. speciosa* are much longer (9 to 15 mm.) than those of *A. syriaca* (less than 7 mm.).

The hybrids could be classed as "*syriaca*-like *speciosa*" and as "*speciosa*-like *syriaca*". The former type resembled *A. syriaca* in regard to leaf size and shape and stem height, but the leaves bore the pubescence of *A. speciosa*. However, judging by the hood length (9 to 10 mm.), this type was to be placed in the latter species.

The "*speciosa*-like *syriaca*" plants were similarly placed within the species *syriaca* on the basis of hood size. They resembled *A. speciosa* in leaf shape and texture and in the lesser stem height.

Cross-pollinations of these possible hybrids with more typical individuals of each species were attempted; the results are shown in Table II.

TABLE II

RESULTS OF ARTIFICIAL POLLINATIONS OF TYPICAL *A. speciosa* AND *A. syriaca* WITH INTERGRADING FORMS OF BOTH SPECIES

| Pollination | No. fls. pollinated | No. pods obtained |
|---|---|---|
| *A. syriaca* (*speciosa*-like) × *A. speciosa* | 7 | 1 |
| *A. speciosa* (*syriaca*-like) × *A. syriaca* | 4 | 0 |
| *A. speciosa* (*syriaca*-like) × *A. syriaca* (*speciosa*-like) | 4 | 2 |
| *A. speciosa* × *A. syriaca* (*speciosa*-like) | 11 | 2 |
| *A. speciosa* (*syriaca*-like) × *A. speciosa* | 3 | 0 |

Similar supposed hybrids between these species were mentioned by Stevens (9). Moreover, this author was successful in obtaining seven mature pods from approximately 90 flowers of *A. syriaca* pollinated artificially with pollen of *A. speciosa.*

Possible hybrids of *A. incarnata* with the variety *pulchra* and the form *albiflora* were grown from seed gathered from plants in experimental plots containing these three types.

*Asclepias incarnata* variety *pulchra* (Ehr.) Pers. is distinguished from the species by its broader leaves and greater degree of pubescence on stem and leaf. The stem of the species is glabrous or bears only a slight line of hair. Further, variety *pulchra* has paler flowers, dull rose in colour, in contrast to the bright rose of the species.

These apparently hybrid plants showed the *pulchra* flower colour associated with the relatively glabrous stem and the leaf characters of the species. Varying degrees of stem pubescence also occurred. The association of the *incarnata* flower colour with the pubescence and leaf shape of the variety has not been seen. Seed of a single pod frequently produced a population including individuals with *pulchra* flower colour and glabrous stem and other plants completely typical of the species.

The form *albiflora* differs from the species only in pigmentation. The flowers of the former are white and pigment is wholly lacking in stems and flower peduncles and pedicels. Apparent hybrids between this form and the species also were found. Seed of a single pod of an *A. incarnata* plant sometimes produced plants that appeared typical and others that lacked pigment and were identical with the form *albiflora.*

The cross *A. incarnata* f. *albiflora* × *A. incarnata* was made artificially. All individuals (11) of the $F_1$ generation showed the bright rose flower colour and rose-purple stem pigmentation of *A. incarnata.* Presumably flower colour and stem pigmentation is genetically dominant to the lack of these characters.

This conclusion was further supported by the ratio between the number of white and of coloured individuals in the natural hybrid populations referred to above.

From these observations it seems probable that the three forms of this species can be hybridized and that difficulties in technique were the cause of the failure of some of the cross-pollinations attempted.

Two plants were observed that apparently were chimaeras of tissue of *A. incarnata* and of the form *albiflora*. In these, several pigmented stems bearing coloured flowers and, as well, some unpigmented stems with white flowers arose from a single root. Examinations of leaf smears from the two kinds of stems revealed that the chromosome number was the same in both, $2n = 22$. It seems therefore that the colour difference was caused by a gene mutation rather than by either a duplication or loss of a whole chromosome in either form. These plants were grown from seed collected from plots of *A. incarnata* f. *albiflora*. It is quite possible that the seed plants had been crossed by insect activity with nearby plants of *A. incarnata*. In this case it would be expected that the seed would produce heterozygous plants showing the dominant characters of the species. It is therefore impossible to say whether the mutation produced *albiflora* stems from pigmented tissue or the reverse. Only four plants survived to maturity in this plot, the other two being phenotypically *A. incarnata*. A view of the root of one of these plants, showing three unpigmented and four pigmented stems, is seen in Fig. 12.

*Self-fertility*

Self-pollination of *A. syriaca* and of *A. incarnata* was attempted but always without success. That self-fertile plants of both species exist has been reported by Plotnikova (8) and Stevens (9) for *A. syriaca*, and for *A. incarnata* by Fischer (3).

## Acknowledgments

A considerable part of the intraspecific pollinations of *A. syriaca* was done by Miss M. K. Osler, summer assistant. The writer wishes to express his gratitude for this assistance. He is indebted also to Dr. Margaret Landes and Dr. H. A. Senn for assistance and criticism in the preparation of the manuscript. Determinations of *A. syriaca* and *A. speciosa* were made with the aid of a new key prepared by Mr. M. N. Zinck.

## References

1. EICHHORN, A. and FRANQUET, R. Numération chromosomique et étude de la mitose somatique chez *Asclepias cornuti*. Compt. rend. 202 : 776-778. 1936.
2. FINN, W. W. Male cells in Angiosperms. I. Spermatogenesis and fertilization in *Asclepias cornuti*. Botan. Gaz. 80 : 1-25. 1925.
3. FISCHER, E. Der Anbau einer neuen Faser- und Bienenfutterpflanze. Pflanzenbau, 17 : 212-218. 1941.
4. FRYE, T. C. Development of the pollen in some Asclepiadaceae. Botan. Gaz. 32 : 325-331. 1901.
5. FRYE, T. C. A morphological study of certain Asclepiadaceae. Botan. Gaz. 34 : 389-413. 1902.

PLATE I

FIG. 12. *Root and lower part of stems of a chimaeral plant of Asclepias incarnata L. showing three unpigmented and four pigmented stems.*

6. GAGER, C. S. The development of the pollinium and sperm cells in *Asclepias cornuti* Dcne. Ann. Botany, 16 : 123-148. 1902.

7. MOYER, L. S. Chromosome numbers and electrophoresis of latex in *Asclepias*. Botan. Gaz. 97 : 860-865. 1936.

8. PLOTNIKOVA, T. An experiment in self-pollination on *Asclepias cornuti*. Ukraine Acad. Sci., Inst. Botan. J. No. 26-27 : 127-135. 1938. Author's English summary.

9. STEVENS, O. A. The cultivation of milkweed. N. Dak. Coll. Agr. Expt. Sta. Tech. Bull. 333. 1945.

10. STEVENS, W. C. The behaviour of kinoplasm and nucleolus in the division of the pollen mother cells of *Asclepias cornuti*. Kansas Univ. Quart. 7 : 77-86. 1898. (Only Abstract seen.)

11. STRASBURGER, E. Einige Bemerkungen zu der Pollenbildung bei *Asclepias*. Ber. deutsch. botan. Ges. 19 : 450-461. 1901.

# NOTES ON SEED-BORNE FUNGI

## IV. *ACREMONIELLA, CHLAMYDOMYCES,* AND *TRICHOCLADIUM*[1]

By J. W. Groves[2] and A. J. Skolko[3]


### Abstract

Two species of *Acremoniella*, *A. atra* (Cda.) Sacc. and *A. verrucosa* Togn., one species of *Chlamydomyces*, *C. palmarum* (Cke.) Mason, and one species of *Trichocladium*, *T. asperum* (Cda.) Harz, have been isolated from agricultural seeds and are described and illustrated. These fungi do not appear to be of serious pathological significance.


### The genus *Acremoniella*

The genus *Acremoniella* was erected by Saccardo (12) as a segregate from *Acremonium* Link and was said to differ from *Acremonium* in having coloured spores. Seven species were listed of which the second, *A. atra* (Cda.) Sacc., should be regarded as the type. Mason (11) has pointed out that the first species, *A. fusca* (Kze. & Schm.) Sacc., has not been recognized with certainty since, and that *A. atra* was the only species that Saccardo had studied himself. *A. atra* is the commonest and best known species and its recognition as the type of the genus would fix the generic name in the sense commonly used.

The genus is characterized by the large, brown, one-celled, ovoid to subglobose spores borne singly on the tips of pointed, septate, branched conidiophores. In the terminology used by Mason (11) these spores are considered to be aleuriospores, or essentially terminal chlamydospores. He also described phialospores but, in the light of later knowledge of sexuality in the ascomycetes, these should probably be interpreted as spermatia. The perfect stage has not been established, but Mason suggested that it might prove to be a *Melanospora*. No perfect stage has appeared in any of our cultures.

We have isolated two species of *Acremoniella* from seeds, *A. atra* (Cda.) Sacc. and *A. verrucosa* Togn.

***Acremoniella atra*** (Corda) Sacc. Syll. Fung. 4 : 302. 1886.

This is the best known species of the genus, and the concept of the species appears to be clear-cut but it raises an interesting problem in nomenclature.

The specific name is based on *Acremonium atrum* Corda published in 1837; consequently the validity of Saccardo's combination, *Acremoniella atra*, rests on whether or not Saccardo correctly identified his fungus as *Acremonium atrum* Corda. Mason was unable to locate any type specimen of *A. atrum* Cda. and argued that if no Corda specimens existed, it was possible to accept

[1] *Manuscript received February 7, 1946.*

[2] *Contribution No. 846 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.*

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Saccardo's determination and recognize Corda's specific name as valid. This has the advantage of retaining a well-known name but raises the question as to what is to be considered the type of the species.

The International Rules provide that a figure may be taken as the type when no specimen exists. Corda's figure in Pl. 3, Fig. 68, of the Icones, labelled *Acremonium atrum*, illustrates a fungus with spores somewhat similar in shape to those of *Acremoniella atra* as commonly conceived, but borne on somewhat different conidiophores. In this figure the conidiophores are long, unbranched, with rather numerous, close septa, and tapering gradually and evenly from the base to the tip. This is in contrast to the rather sparingly septate conidiophores of *Acremoniella*, which branch frequently at right angles or nearly so, and usually taper gradually near the base and more abruptly toward the tip. It would appear that Corda's figure could only be accepted as the type of Saccardo's fungus by assuming that Corda drew the conidiophores inaccurately.

However, not only Corda's figure but also his description does not fit our fungus. His choice of the specific name "*atra*" would imply that the fungus was black, and Mason (11) pointed out that Corda's description stated that the thallus and spore contents were very black, but *Acremoniella atra* in Saccardo's sense is not black.

Thus there is good reason to believe that Saccardo's identification of his fungus as *Acremonium atrum* Corda was incorrect, and that consequently the use of the specific name "*atra*" for this fungus is not permissible. According to the synonymy proposed by Mason (11), the next available name is *Monosporium acremonioides* Harz, published in 1871. A new combination in *Acremoniella* based on this species would, therefore, probably provide the correct name for our fungus, and Harz's material would become the type. Unfortunately, no material of *Monosporium acremonioides* is available at present to establish this identification, and to create a new combination now would be merely substituting one guess for another. Furthermore, since Mason's paper was published, at least part of Corda's herbarium has been discovered in Prague and it is possible that a Corda specimen may yet come to light that will establish beyond question the identity of *Acremonium atrum*.

Until such time as the Corda and Harz specimens can be further studied we have, therefore, decided to use the well-known name *Acremoniella atra* for this fungus. Probably the name will have to be changed, but this should only be done after comparison with authentic material of *Monosporium acremonioides*. In the meantime, the use of a well-known name, even though incorrect, seems preferable to creating a new combination based merely on a supposition.

*A. atra* appears to be widely distributed on various substrata and is fairly well-known. Mason (11) described the growth in culture quite adequately, and gave a list of synonyms. We have never isolated it directly in pure culture from seeds but it has been invariably associated with *Alternaria tenuis* auct. It was easily obtained in pure culture by picking off the large spores.

The spores are golden brown or cinnamon brown, one-celled, ovoid to subglobose, usually smooth walled, sometimes with a slight wrinkling of the exospore, (19)-22-28-(32) × (16)-18-24-(26)$\mu$ (Figs. 1, 5). The conidiophores are hyaline, rather sparingly septate, simple or branching at right angles or nearly so, straight or slightly curved, variable in length, 4–8$\mu$ in diameter at the base, tapering to a slender tip where the spores are borne. The microconidiophores are hyaline, septate, variable in length, aspergilliform with a terminal vesicle about 12$\mu$ in diameter bearing flask-shaped phialides about 4$\mu$ in diameter at the base and 6$\mu$ in length. The microconidia are hyaline, ovoid to globose, 1.5–2.0$\mu$ in diameter, borne in chains.

It has been isolated from the following seeds: *Allium cepa* L. (onion), *Beta vulgaris* L. (beet), *Daucus carota* L. var. *sativa* DC. (carrot), *Festuca rubra* L. (red fescue), *Lolium perenne* L. (rye grass), *Pastinaca sativa* L. (parsnip), *Pisum sativum* L. (peas), *Raphanus sativus* L. (radish), *Trifolium hybridum* L. (alsike clover), *Zea mays* L. (corn).

It has been isolated from seeds from Ontario, British Columbia, Denmark, and Holland.

As noted above, we have always found *A. atra* associated with *Alternaria tenuis* auct. when isolated from seeds. Under these conditions it grows rapidly, producing a luxuriant development of aerial mycelium and abundant spores. When grown in pure culture, however, it grows much more slowly, produces less mycelium and fewer spores.

In order to find out whether the difference in growth rate was due to the presence of the *Alternaria*, Petri dishes were inoculated at opposite sides with cultures of *Acremoniella* and *Alternaria*. Malt extract agar and potato dextrose agar were used. Growth was uniform in both fungi until the colonies met, when a pronounced stimulation of growth occurred in the *Acremoniella*. From the point of contact of the two mycelia, the hyphae of the *Acremoniella* quickly spread over and through the *Alternaria* colony. There was no corresponding stimulation of the *Alternaria* and hyphae of the *Acremoniella* that did not actually come into contact with the *Alternaria* did not exhibit any stimulation. The same effect was observed when *A. atra* was grown in association with *Fusarium moniliforme* Sheldon and *Cephalosporium acremonium* Cda.

Mason (11) observed that *A. atra* would not grow in pure culture on Dox agar, but would grow in association with *Cephalosporium acremonium* Cda. We repeated this experiment and obtained the same result with an isolate of *C. acremonium* from corn, but with an isolate of *C. acremonium* from peas, which was morphologically indistinguishable from the corn isolate, it refused to grow.

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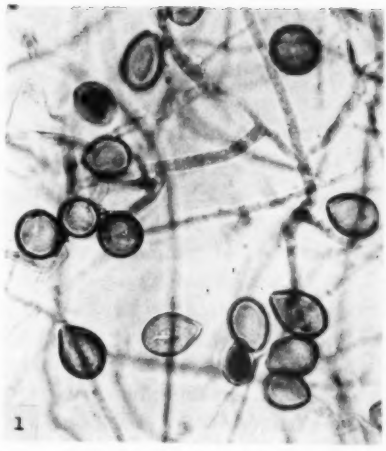
FIG. 1. *Microphotograph of Acremoniella atra (Corda) Sacc.* FIG. 2. *Microphotograph of Acremoniella verrucosa Togn.* FIG. 3. *Microphotograph of Chlamydomyces palmarum (Cooke) Mason.* FIG. 4. *Microphotograph of Trichocladium asperum (Cda.) Harz.*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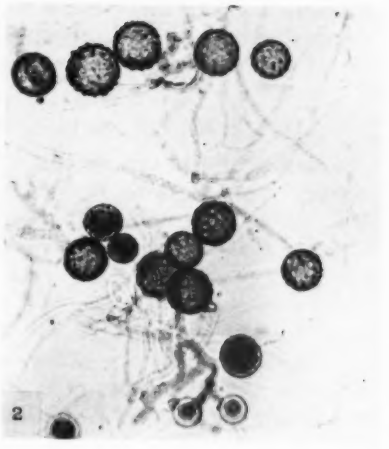
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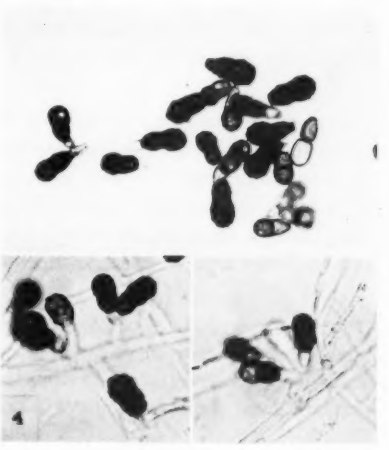
3


4

It is evident that the presence of other fungi may have a decided effect on the growth of *A. atra*, not only in the synthetic media such as Dox agar, but also in carbohydrate media such as malt extract agar, pea decoction agar, and potato dextrose agar. Nothing is known of the reason for this reaction but several interesting problems are suggested by it. The fungus is generally regarded as a saprophyte but it would be desirable to investigate the possibility that if it is pathogenic, its pathogenicity might also be affected by the presence of other fungi.

***A. verrucosa*** Togn. Rend. ist. lombardo sci., 2 ser., 29 : 864. 1896.

This species can be distinguished from *A. atra* by the rough-walled, slightly smaller, and more globose spores. They are light brown, ovate to globose, one-celled, verrucose to tuberculate, 17–26 × 17–23$\mu$ (Figs. 2, 6). The conidiophores are hyaline, septate, simple or usually branched at right angles or nearly so, variable in length, 5–8$\mu$ in diameter at the base, tapering to a slender tip on which the spore is borne. The microconidiophores are aspergilliform, hyaline, septate, not branched, variable in length, about 5–6$\mu$ in diameter, swollen at the tip into a vesicle 10–13$\mu$ in diameter on which flask shaped to ovoid phialides, 4–5$\mu$ in diameter at the base and 4–10$\mu$ in length, are borne. The microconidia are hyaline, one-celled, broadly ellipsoid to ovoid, catenate, 2.5–3.0 × 1.5–2.0$\mu$.

This species has only been isolated on two occasions, once from seed of *Pisum sativum* L. from Ontario and once from seed of *Taraxacum kok-saghyz* Rod. from the U.S.S.R.

Horne and Jones (7) who studied *A. verrucosa* and *A. atra* under the names *Eidamia tuberculata* and *E. acremonioides* respectively, considered that in addition to their morphological differences, they were distinct physiologically in the ability of *A. verrucosa* to utilize certain media that *A. atra* could not. Mason (11) questioned this because he found, from a study of the type culture of *Eidamia tuberculata*, that it behaved like a mixture of *A. atra* and *Cephalosporium* on Dox agar.

When grown on Dox agar in pure culture, our isolates of *A. verrucosa* also failed to grow. In association with *Alternaria tenuis* or *Fusarium moniliforme*, they grew and sporulated profusely, but with *Cephalosporium acremonium* growth was slight. This confirms Mason's findings that *A. atra* and *A. verrucosa* behave similarly on Dox agar.

## The Genus *Chlamydomyces*

The genus *Chlamydomyces* was erected by Bainier (1) and was based on a fungus that he described as *C. diffusus*. This fungus, collected on cow dung, was said to have the mycelium and conidiophores of *Acremoniella atra* but the spores of *Mycogone cervina*. He considered that it differed from *Mycogone* in its saprophytic rather than parasitic habit of growth and in its lack of a microconidial stage.

Later work has shown that the latter distinction does not hold. Hotson (8) reported microconidia in *Mycogone Ulmariae* Potebnia, which he considered to be a synonym of *Chlamydomyces diffusus*. Mason (10) also described microconidia in *Chlamydomyces* but thought the genus should be accepted and could be distinguished from *Mycogone* by the cuneate rather than subglobose basal cell of the spore, and by the lack of constriction at the septum. He was of the opinion that species of *Mycogone* were parasites of other fungi and that all records of *Mycogone* on flowering plants should probably be referred to *Chlamydomyces*.

The similarity between *Chlamydomyces* and *Acremoniella* has been apparent to all who have studied these species. They have the same type of microconidial fructification and similar growth habit, and differ chiefly in the two-celled spores of *Chlamydomyces*. There is some evidence that the small basal cell of the *Chlamydomyces* spore is in reality the tip of the conidiophore, and, if this is the correct interpretation, as seems probable, even this distinction breaks down. The two genera are undoubtedly very closely related. The perfect stage of *Chlamydomyces* is still unknown. Only the one species has been isolated from seeds.

***C. palmarum*** (Cooke) Mason. Annotated account of fungi received at the Imp. Bur. Mycol. List 2, Fasc. 1 : 37. 1928.

This species was originally described by Cooke (3) as *Trichobasis palmarum* on leaves of *Cocos nucifera* and was transferred to *Chlamydomyces* by Mason (10) after comparison of living cultures with authentic specimens in Kew Herbarium. Mason was unable to locate any type material of *C. diffusus* Bainier, the type of the genus, but there seems little doubt that it is the same fungus. Our isolates agree well with the descriptions and one was sent to Mr. Mason, who kindly confirmed the identification.

The spores are ovoid, narrowed toward the base, two-celled, not constricted at the septum, the upper cell yellow, verrucose, and much larger, the lower cell hyaline, smooth, cuneate, and much smaller, 28–50 × 22–40$\mu$ (Figs. 3, 8). The conidiophores are hyaline, septate, simple or branched at right angles or nearly so, variable in length, 7–10$\mu$ in diameter at the base, tapering to a slender tip. The microconidiophores are hyaline, septate, simple, variable in length, 4–5$\mu$ in diameter, aspergilliform, the terminal vesicle 8–12$\mu$ in diameter and bearing flask-shaped phialides 4–5$\mu$ in diameter at the base and 7–13$\mu$ long, the phialides occasionally produced singly on the hyphae. The microconidia are hyaline, one-celled, oblong to ellipsoid, 3–5 × 1.5–2.0$\mu$, borne in chains.

It has only been isolated from seeds on two occasions, once from *Pastinaca sativa* L. (parsnip), and once from *Pisum sativum* L. (peas). The seed of both samples was produced in Ontario.

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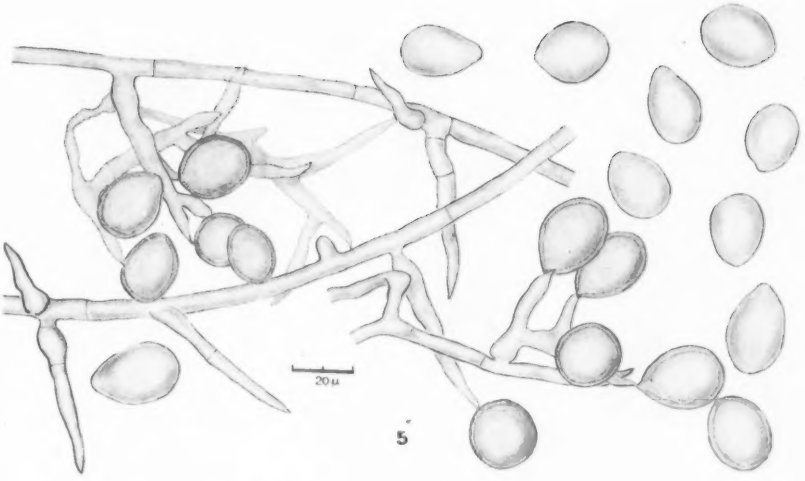
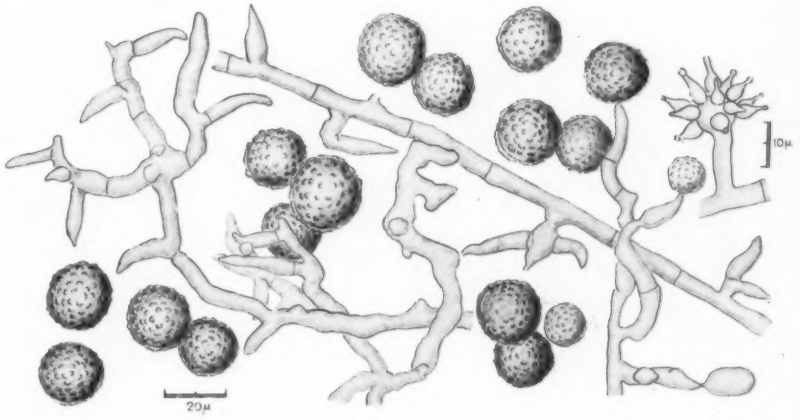
FIG. 5. *Acremoniella atra (Corda) Sacc.* FIG. 6. *Acremoniella verrucosa Togn.* FIG. 7. *Trichocladium asperum (Cda.) Harz.* FIG. 8. *Chlamydomyces palmarum (Cooke) Mason.*

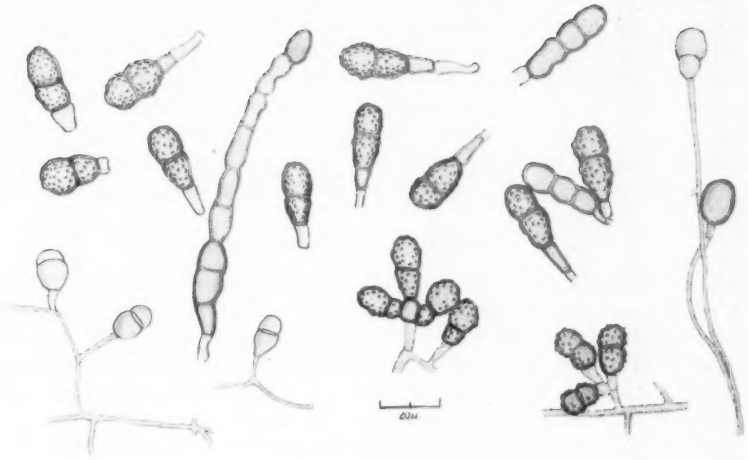
PLATE II



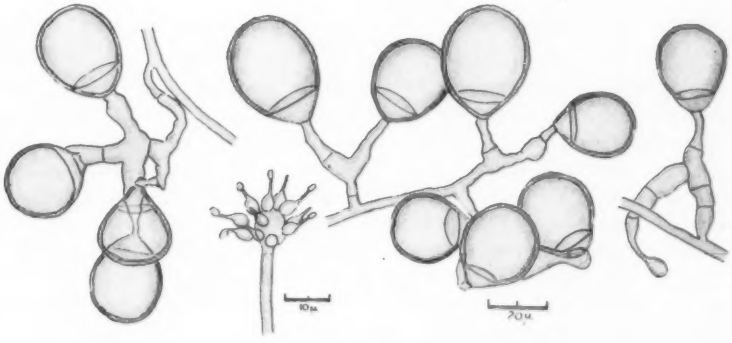

5




6




7




8

This species sporulates well and produces a more luxuriant growth of mycelium on malt extract agar than either of the *Acremoniella* species.

On Dox agar some growth occurred but it was very markedly reduced as compared to the growth on malt. A few rather coarse hyphae were produced that grew in a straggling fashion over the plate with few branches or aerial hyphae and very scant spore production. When grown with *Cephalosporium acremonium* on the same medium there was some evidence of stimulation but it was slight. However, with *Alternaria tenuis* and *Fusarium moniliforme* it grew luxuriantly and produced abundant aerial mycelium and spores. The similarity of this behaviour to that of the *Acremoniella* species would further indicate a close relationship between them.

## The Genus *Trichocladium*

The genus *Trichocladium* was erected by Harz (6) based on a fungus that he considered to be *Sporidesmium asperum* Corda. The spores are two-celled, dark, rough-walled, borne singly on the tips of short conidiophores. It seems to be close to the older genus *Dicoccum* Corda, and some authors, Lindau (9), Clements and Shear (2), and Gilman (5), have considered it to be a synonym. However, Mason (11) has suggested that because the structure of the type of *Dicoccum* is obscure and Corda himself did not recognize *Sporidesmium asperum* as a species of his genus *Dicoccum*, it would be advisable to retain *Trichocladium* for the present. Only one species has been isolated from seeds.

***Trichocladium asperum*** (Cda.) Harz. Bull. soc. imp. naturalistes Moscou, 44 : 125. 1871.

On malt extract agar different isolates vary to some extent in colour and amount of aerial mycelium. They may be dark olive grey to iron grey or dark greenish olive to light yellowish olive, to olive yellow or olive ochre (Ridgway). The conidiophores are hyaline, simple or occasionally branched, septate, 3–4$\mu$ in diameter, variable in length, 10–56$\mu$ or more. The conidia are oblong-ellipsoid to clavate, at first hyaline, becoming brown and finally nearly black, two-celled, slightly constricted at the septum, the upper cell rounded, the lower cell narrowed toward the base, at first slightly smaller than the upper cell, later about the same size, tuberculate, (14)–16–22–(25) $\times$ (10)–11–14–(15)$\mu$ (Figs. 4, 7). Occasionally the spores become three-celled and sometimes one-celled spores are formed. The latter are ovoid to subglobose and about the same size as a single cell of the normal two-celled spores. Frequently a small piece of the end of the conidiophore remains attached to the spore, appearing as a short pedicel. No microconidia have been observed.

It has been isolated from seeds of *Cucurbita Pepo* L. (pumpkin), *Daucus carota* L. var. *sativa* DC. (carrot), *Lactuca sativa* L. (lettuce), *Pisum sativum* L. (peas), and *Vicia faba* L. (broad beans). It has been isolated from seeds from British Columbia, Connecticut, and Missouri.

In his account of *T. asperum*, Harz (6) expressed some doubt as to the correctness of his identification of this fungus as *Sporidesmium asperum* Cda. He pointed out some discrepancies between Corda's description and figure but concluded that if the characters of both description and figure were taken, it was probably the same as his fungus. Dufour (4) described the growth in culture and the way in which the spores are formed. He was of the opinion that there was less difference between the figures of Corda and of Harz than between cultures of the same strain grown on sterilized orange juice and a sugar solution, and that Harz's identification was probably correct. It would seem, therefore, that this name may be accepted as valid unless someone can show from an examination of Corda's material that *Sporidesmium asperum* was a different fungus.

This species exhibited no stimulation of growth when grown with *Alternaria tenuis*, *Cephalosporium acremonium*, or *Fusarium moniliforme*, and it grew quite well in pure culture on Dox agar. The appearance of the cultures and manner of growth is very different from cultures of *Acremoniella* and *Chlamydomyces*. In spite of the superficial similarity of dark, rough-walled spores, it seems probable that when the perfect stages of these forms are eventually discovered, *Trichocladium* will prove to be not closely related to the other two genera.

## Acknowledgments

The authors are indebted to Mr. E. W. Mason, Imperial Mycological Institute, and Dr. D. H. Linder, Farlow Herbarium, Harvard University, for assistance in the identification of these fungi.

## References

1. BAINIER, M. G. Mycothèque de l'Ecole de Pharmacie de Paris. XXIII. Les genres *Hypomyces*, *Trichocladium*, et *Acremoniella* comparés au genre nouveau *Chlamydomyces*. Bull. trimestr. soc. mycol. France, 23 : 235-241. 1907.
2. CLEMENTS, F. E. and SHEAR, C. L. The genera of fungi. The H. W. Wilson Co., New York. 1931.
3. COOKE, M. C. Cocoa-palm fungi. Grevillea, 5 : 101-103. 1877.
4. DUFOUR, L. Observations sur le développement et la fructification du *Trichocladium asperum* Harz. Bull. soc. bot. France, 35 : 139-144. 1888.
5. GILMAN, J. C. A manual of soil fungi. The Collegiate Press, Inc., Ames, Ia. 1945.
6. HARZ, C. O. Einige neue Hyphomyceten Berlins und Wiens nebst Beiträgen zur Systematik derselben. Bull. soc. imp. naturalistes Moscou, 44 : 88-147. 1871.
7. HORNE, A. S. and JONES, G. H. A further contribution to the morphology and physiology of the genus *Eidamia*. Ann. Botan. 38 : 351-359. 1924.
8. HOTSON, J. W. Culture studies of fungi producing bulbils and similar propagative bodies. Proc. Am. Acad. Arts Sci. 48 : 227-306. 1912.
9. LINDAU, G. Fungi imperfecti : Hyphomycetes. *In* Rabenhorst, Kryptogamen-Flora von Deutschland, Österreich, und der Schweiz, I, 8 : 753-851. 1907.
10. MASON, E. W. Annotated account of fungi received at the Imperial Bureau of Mycology, List II, Fasc. 1. 1928.
11. MASON, E. W. Annotated account of fungi received at the Imperial Bureau of Mycology, List II, Fasc. 2. 1933.
12. SACCARDO, P. A. Sylloge fungorum. IV. Patavii. 1886.

# TETRAPLOID *TARAXACUM KOK-SAGHYZ*

## II. CHARACTERS OF $F_1$ PLANTS GROWN IN POTS[1]

By M. W. Bannan[2]


## Abstract

$F_1$ tetraploids grown in pots had larger but fewer leaves and inflorescences than control diploids. Increase in size of the organs was offset by reduction in number, so that leaf area was no greater and total number of florets per plant was much lower in tetraploids than in diploids. Fertility varied inversely with genetic relationship and was much reduced in the tetraploids. No significant differences were noted in root size. The tetraploids grew slightly more slowly than diploids but for a longer period, and, on the whole, plants with the largest rosettes developed the biggest roots. Sibling tetraploids exhibited less deviation from the mean than randomized plants in such characters as number, size, and shape of leaves, production of heads, and weight of roots. Leaf characteristics provided the clearest evidence of an inheritance mechanism. The data on root weight were less conclusive.


## Introduction

In a previous publication (2) the production of tetraploid kok-saghyz by the colchicine technique was described. Certain characteristics of the $4n$ plants were discussed, but since the majority of the colchicined plants were chimaeras, it was pointed out that the critical determination of the effects of tetraploidy would have to be made from the second colchicine ($C_2$) or first filial ($F_1$) generation. Such plants are described in the present paper and comparison made with diploids grown in parallel series. It should be noted that the descriptions refer to plants grown in pots. Observations on $2n$ and $4n$ kok-saghyz cultivated under agronomic conditions do not come within the scope of this paper.

## Methods

For comparative studies five series of $F_1$ tetraploids and diploids were established, differing in origin of seed and conditions of growth. These series were designated as $A^1$, $B^1$, $C^1$, $D^1$, and $E^1$, but were not necessarily derived from the series of corresponding designation ($A$ to $E$) in the parental generation already described (2). The seed was obtained in two ways. The first lot of $4n$ seed was procured by random, unbagged, and unrecorded crossing of tetraploids in parental Series $C$. The second lot was obtained a few weeks later by controlled, bagged crossing of described and recorded plants in Series $A$, $B$, and $C$. The roots of these parental plants were later weighed and the rubber content of many was determined. The first lot of diploid seed was collected at random from parental Series $C$, the plants of which were colchicined, selected on the stomatal criterion as possible tetraploids, but produced predominantly diploid inflorescences. The second lot of diploid seed was a

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*Contribution from the Department of Botany, University of Toronto, Toronto, Ont., with financial assistance from the National Research Council of Canada.*

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random mixture from untreated field diploids. The origin and handling of the several series are summarized in Table I.

The growing season of 1945 was unusual. Owing to persistent, deep snow cover, the ground was never frozen during the winter. The snow disappeared

TABLE I

ORIGIN AND TREATMENT OF $F_1$ DIPLOIDS AND TETRAPLOIDS

| Series | Purpose | Origin of seed | | Conditions of growth | |
|---|---|---|---|---|---|
| | | $2n$ | $4n$ | Early growth | Subsequent growth |
| $A^1$ | Comparison under suppressed growth of diploids and tetraploids both derived from colchicined parents | Random crosses of colchicined parents | Random crosses | Germinated Aug. 1944. Set out in flats, and later transferred to 4-in. pots | Plunged outside and exposed to ground frost. Returned to greenhouse in Nov. Little winter growth. Repotted in 6-in. pots Mar. 1945. Retained in greenhouse all summer. Growth meagre |
| $B^1$ | Comparison under optimal growth of plants similar to $A^1$ | As above | As above | As above | Plunged outside and there overwintered. Returned to greenhouse Mar. 1945 and repotted in 9-in. pots. Growth rapid and luxuriant. Largest and most floriferous plants occurred here |
| $C^1$ | Comparison under intermediate growth conditions of plants similar to $A^1$ | As above | As above | As above | Plunged outside and there overwintered. After repotting in 8- or 9-in. pots were left to grow out of doors, spring and summer 1945. Growth moderate, flowering later and less than $B^1$. Reaction to summer dormancy more severe |
| $D^1$ | To determine whether $F_1$ tetraploids gave evidence of inheritance mechanism for vegetative and reproductive characters | As above | Recorded crosses between parents with wide range of leaf type, root size, and rubber content | Germinated Mar. 1945. Set out directly in 2-in. pots, then transferred to 8-in. pots | Plunged outside. Growth good, but of one season only |
| $E^1$ | To function as control and to permit cross comparisons with $D^1$ of colchicined diploids, random diploids, random tetraploids, and recorded tetraploids | Random crosses of untreated field plants | Randomized seed of recorded crosses | As above | As above |

during unseasonably warm weather in April. May was excessively wet and cool, severely retarding growth out of doors. Moisture was superabundant throughout June. When drier weather came in August a marked summer dormancy was manifested in the field from which later recovery was but slight. All plants were unearthed in October, 1945. The leaves were removed and the roots washed and weighed. Some were dried and stored for rubber analysis, others were repotted for future crossing.

## Germination and Seedling Development

Germination of the achenes from colchicined parental diploids and tetraploids was retarded and this effect was attributed to the choice of cold and humid rather than warm and dry conditions of winter storage. The germination of seed from untreated field diploids stored at room temperature seemed unaffected. Under the retarded conditions no consistent differences were observed between random $2n$ and $4n$ seed in either rate or amount of germination. In the growth of the seedlings it was noted that the diploids produced more numerous, slightly longer, but definitely narrower leaves.

The controlled $4n$ crosses exhibited wide differences in the rate and amount of germination. The achenes from one seed parent in particular were notable for rapid germination. In certain other cases germination was tardy but ultimately high. Some parental individuals were good both as seed and pollen parents; others were good pollen parents but poor seed parents, or vice versa. Mean weight of the sound achenes varied from 0.52 to 0.98 mgm. in the crosses studied, but weight was not the major factor in determining germination or final size of the plant. Achenes from the upper half of the seed population weighed 0.71 to 0.98 mgm., germinated to 74%, produced rosettes with an average of 29 leaves at the peak of leaf production, and roots weighing 16.4 gm. Achenes from the lower half, weighing 0.52 to 0.71 mgm., germinated to 83% and developed plants of 28 leaves and roots of 19.1 gm.

## Leaves

*Number of Leaves*

Leaf production varied widely, as shown in Table II, but was, on the whole, markedly greater in $2n$ than in $4n$ plants. The first-year diploids (Series $D^1$ and $E^1$), at the point of maximum development, had 84% more *green* leaves than had the corresponding tetraploids, and in second-year plants (Series $B^1$ and $C^1$) the margin was 64%. The differences in total leaf production were much wider than these values indicate, because of the more rapid turnover of leaves in the diploids. In this, as in other growth characteristics, the tetraploids appeared torpid as compared with the diploids.

The greatest leaf production occurred in Series $B^1$ where the pots were largest and the growing season longest. Leaf development was greater also in second-year (Series $B^1$ and $C^1$) than in first-year (Series $D^1$ and $E^1$) plants, especially in the tetraploids. Among the latter, wide differences were noted between the various crosses. For instance, the $F_1$ progeny of the 31 $\times$ 86

developed rosettes with an average maximum of 40 leaves, whereas in the progeny of 71 × 296 and 296 × 71 the average numbers were 24 and 22.

*Length of Leaves*

Employing rosette diameter as the criterion, leaf length was found to vary greatly between individuals, but Table II shows that at the peak of development mean differences between $4n$ and $2n$ plants lay within 11%. Except in

TABLE II

RANGE AND AVERAGE DIAMETER OF THE ROSETTES AND NUMBER OF GREEN LEAVES AT MAXIMUM DEVELOPMENT (1945)

| Series | $2n$ | | | $4n$ | | |
|---|---|---|---|---|---|---|
| | No. of plants | Diam. in in. | No. of leaves | No. of plants | Diam. in in. | No. of leaves |
| $A^1$ | 21 | 5– 9 ( 7.2) | 16– 62 (39) | 36 | 5–10 ( 7.5) | 14– 57 (34) |
| $B^1$ | 18 | 10–14 (12.0) | 30–138 (75) | 36 | 9–18 (12.6) | 20–127 (48) |
| $C^1$ | 34 | 8–14 (10.1) | 27–122 (52) | 66 | 7–14 ( 9.5) | 13– 50 (30) |
| $D^1$ | 42 | 7–13 ( 9.2) | 22– 83 (45) | 548 | 5–14 ( 8.8) | 9– 76 (29) |
| $E^1$ | 32 | 7–12 ( 9.3) | 16–132 (60) | 33 | 5–14 ( 8.4) | 13– 96 (28) |

the suppressed Series $A^1$, the rosettes were wider in second-year (Series $B^1$ and $C^1$) than in first-year plants (Series $D^1$ and $E^1$). The second-year tetraploids slightly exceeded diploids where the pots were biggest and the growth period longest (Series $B^1$). The trend was reversed in first-year plants but the differences were slight. Among the progeny of the recorded tetraploid crosses considerable differences appeared. For example, the average diameter at the peak of development was 10.2 in. in the vigorous progeny of 31 × 86 as compared with 7.0 in. in the progeny of 84 × 86.

*Leaf Width*

This character varied from plant to plant and with stage of development but tended to be greatest among the tetraploids. As noted in the parental generation, certain tetraploids had narrow leaves and simulated diploids, but the majority possessed spatulate early leaves (2). Table III shows the average width of the distal portions of 10 leaves beginning with the widest and longest leaves and including a similar number of smaller but mature leaves formed later. On the whole the leaves were widest in the vigorous second-year plants of Series $B^1$. Measured in April, the mature leaves were 16% wider in $4n$ than in $2n$ plants. Since the widest leaves persisted longer in the tetraploids, their average leaf width was maintained through June whereas the width decreased in the diploids (Table III). The leaves of first-year plants (Series $D^1$ and $E^1$) were narrower, especially among the diploids, and the differences between the $2n$ and $4n$ plants were more pronounced. Under similar conditions of growth the progeny of colchicined diploids (Series $D^1$) had slightly wider leaves than the progeny of untreated field diploids (Series

TABLE III

AVERAGE WIDTH IN INCHES OF THE DISTAL PORTION OF MATURE LEAVES IN THE FULLY EXPANDED ROSETTE

| Series | Date | 2*n* | 4*n* |
|---|---|---|---|
| $B^1$ | April | 0.87 | 1.01 |
| | June | 0.76 | 1.02 |
| $D^1$ All plants | July | 0.65 ± .06 | 0.93 ± .10* |
| Cross 21 × 296 | July | — | 0.92 ± .11 |
| 296 × 21 | July | — | 0.85 ± .05 |
| 354 × 296 | July | — | 1.02 ± .10 |
| 296 × 354 | July | — | 1.05 ± .10 |
| 71 × 17 | July | — | 1.05 ± .10 |
| 71 × 86 | July | — | 0.99 ± .16 |
| 86 × 71 | July | — | 1.03 ± .13 |
| 31 × 21 | July | — | 0.82 ± .09 |
| 21 × 31 | July | — | 0.75 ± .09 |
| 619 × 629 | July | — | 0.78 ± .12 |
| $E^1$ | July | 0.59 ± .12 | 0.83 ± .13 |

* *Mean and standard deviation.*

$E^1$). The first selection of parental colchicined diploids as possible tetraploids was based upon their thicker, wider leaves (2), and this characteristic was maintained in the $F_1$ plants.

The tetraploids of Series $D^1$ provided an even clearer demonstration of an inheritance mechanism. Here the $F_1$ sister plants from controlled crosses were usually remarkably similar in their leaf characteristics while differing from the progeny of other crosses (Table III). The sharp delimitation of the various $F_1$ families as viewed in the garden plot was most striking. For example, the progeny of 31 × 21 and the reciprocal of 21 × 31 had deeply cut leaves averaging 0.82 and 0.75 in. wide, whereas the plants of 71 × 86 and its reciprocal possessed more or less entire leaves 0.99 and 1.03 in. wide.

No measurements were made of total leaf area but it was evident that the greater width of tetraploid leaves did not compensate for their markedly smaller numbers. Estimates from the available data indicated that the ratio of 4*n* to 2*n* leaf area was only about 1 : 1.6 in the early July of the first year (Series $E^1$). Since the tetraploids matured later than the diploids and the largest of the former did not complete growth until some time after the diploids, the differences in leaf area narrowed as the season progressed. In April and May of the second year (Series $B^1$) the ratio of 4*n* to 2*n* leaf area was 1 : 1.1.

*Leaf Margin*

Some plants produced more or less entire leaves throughout the growing period while in others the late spring and early summer leaves were increasingly dissected. For descriptive purposes the various kinds of leaves were classified into five types slightly differing from the five (*A* to *E*) described by Miss

Kern (6) for *Taraxacum palustre* var. *vulgare* and *T. laevigatum*. These are: Type I, margins entire or nearly so; Type II, sinuses less than one-fourth the leaf width; Type III, sinuses one-fourth to one-half the leaf width; Type IV, sinuses one-half to three-fourths the leaf width, and Type V, sinuses exceeding three-fourths the leaf width, extending almost to the midrib. The distribution of leaf types among the first-year population is shown in Table IV. On the

TABLE IV

DISTRIBUTION OF LEAF TYPES IN FIRST-YEAR PLANTS

| Series | Description | Location | Leaf types of parents | Distribution of $F_1$ plants according to leaf type, % | | | | | Number of $F_1$ plants |
|---|---|---|---|---|---|---|---|---|---|
| | | | | I | II | III | IV | V | |
| $D^1$ | $2n$ | In pots | — | 0 | 10 | 47 | 43 | 0 | 42 |
| | $4n$ All plants | In pots | — | 2 | 26 | 41 | 31 | 0 | 393 |
| | Cross 71 × 17 | In pots | II, II–III | 0 | 76 | 24 | 0 | 0 | 29 |
| | 354 × 296<br>296 × 354 | In pots | II, II-III | 5 | 44 | 30 | 21 | 0 | 43 |
| | 17 × 296<br>296 × 17 | In pots | II–III, II–III | 0 | 38 | 52 | 10 | 0 | 40 |
| | 71 × 86<br>86 × 71 | In pots | II, II | 0 | 28 | 65 | 7 | 0 | 43 |
| | 17 × 31<br>31 × 17 | In pots | II–III, IV | 5 | 33 | 54 | 8 | 0 | 39 |
| | 71 × 296<br>296 × 71 | In pots | II, II–III | 4 | 25 | 46 | 25 | 0 | 24 |
| | 296 × 21<br>21 × 296 | In pots | II–III, III–IV | 0 | 3 | 30 | 67 | 0 | 33 |
| | 21 × 31<br>31 × 21 | In pots | III–IV, IV | 0 | 2 | 24 | 72 | 2 | 46 |
| | 619 × 629<br>629 × 619 | In pots | IV, IV–V | 0 | 0 | 21 | 79 | 0 | 19 |
| $E^1$ | $2n$ | In pots | — | 0 | 6 | 28 | 60 | 6 | 32 |
| | $4n$ | In pots | — | 6 | 17 | 31 | 40 | 6 | 35 |
| Field | $2n$ | Field | — | 1 | 5 | 19 | 61 | 14 | 197 |
| | $4n$ | Field | — | 9 | 19 | 28 | 45 | 4 | 210 |

whole the incidence of deeply dissected leaves (Types IV and V) was lower in tetraploids than in diploids. Differences of similar order were observed in both potted (Series $E^1$) and field plants. It is noteworthy that potted and field plants grown from similar seed did not differ significantly in their leaf types, diploids and tetraploids exhibiting a similar margin of difference in both cases. The $F_1$ progeny of colchicined diploids, selected as possible tetraploids (Series $D^1$) were not quite so deeply cut as the progeny of random untreated diploids (Series $E^1$) and in this respect resembled the parental types.

Of the tetraploids derived from controlled crosses (Series $D^1$) the sister plants in each $F_1$ family were remarkably alike and resembled their parents when both these had the same type of leaf margin. For example, the progeny of 71 × 17 (Table IV) had shallowly cut leaves mostly of Type II, like the

parents. The parental plants 619 and 629 possessed leaves of Type IV and again the progeny resembled the parents, 79% possessing leaves of Type IV (Table IV). When the parents differed markedly in leaf type the progeny were more variable. Thus the cross 17 × 31, with parental leaf Types II and IV, produced progeny with 5% Type I, 33% Type II, 54% Type III, and 8% Type IV.

*Summer Dormancy*

Random second-year diploids and tetraploids showed no marked differences in the rosette during summer dormancy, but wide differences occurred between the differently treated series. The plants of Series $A^1$ resided in the greenhouse throughout winter and summer and cycles of leaf production and flowering followed each other with little evidence of dormancy in either diploids or tetraploids. But in Series $C^1$, the plants of which overwintered outside and remained in the garden plot during the summer, both $2n$ and $4n$ rosettes almost completely disappeared in a profound dormancy. Though their behaviour was variable, dormancy was more marked among first-year diploids than first-year tetraploids (Series $D^1$ and $E^1$). The $F_1$ progeny of the controlled $4n$ crosses varied greatly, the rosettes of some disappearing almost entirely while other plants grew on to well above the average size.

*Stomatal Dimensions*

Measurements were made as for the parental generation (2). Although the range was great, guard cells were on the average 38% longer in tetraploids and the stomata only half as frequent as in diploids (Table V). No outstand-

TABLE V

LENGTH OF GUARD CELLS AND FREQUENCY OF STOMATA

| Series | No. of plants | Range and average length of guard cells ($\mu$) | | Range and average frequency of stomata (per sq. mm.) | |
|---|---|---|---|---|---|
| | | $2n$ | $4n$ | $2n$ | $4n$ |
| $A^1$ | 43 | 26–32 (28.5) | 32–47 (38.5) | 93–166 (129) | 53–117 (75) |
| $B^1$ | 50 | 26–31 (28.5) | 35–45 (40.0) | 106–180 (134) | 43– 90 (59) |
| $D^1$ | 112 | 26–36 (30.0) | 33–46 (41.0) | 84–201 (133) | 54–116 (79) |
| $E^1$ | 63 | 26–35 (30.0) | 37–46 (41.0) | 108–217 (155) | 45–115 (70) |

ing differences were observed between the various series or between first-year and second-year plants. The marked reduction in size of the stomata in the diploids of Series $D^1$ as compared with the colchicined parents, which had predominantly diploid inflorescences but were originally selected as possible tetraploids because of their larger stomata, is doubtless evidence that the parental plants were chimaeras with some $4n$ tissue in their leaves. Among both $2n$ and $4n$ plants there was surprisingly little difference between the depauperate plants of Series $A^1$ and the vigorous plants of Series $B^1$ in

stomatal dimensions. An attempt was made to determine whether a correlation existed between cell size, as indicated by size of stomata, and weight of roots, but because of variation among leaves of the same plant the data proved insufficient to be conclusive.

## Inflorescences

*Head Production*

Table VI records productivity of diploids and tetraploids from early spring to Aug. 31, 1945. In each of the series the diploids were much more floriferous than the tetraploids. Considerable interserial differences also appeared. Maximum flowering took place in the second-year plants of the optimal Series $B^1$, and production was less profuse in the first-year plants of Series $D^1$ and $E^1$, especially among the tetraploids.

TABLE VI

TOTAL HEAD PRODUCTION JAN. TO AUG. 31, 1945

| Series | 2*n* | | 4*n* | |
|---|---|---|---|---|
| | No. of plants | Range and av. no. of heads | No. of plants | Range and av. no. of heads |
| $A^1$ | 22 | 0– 48 (20) | 37 | 0–33 (11) |
| $B^1$ | 18 | 20–123 (64) | 38 | 1–54 (22) |
| $C^1$ | 35 | 12–111 (48) | 68 | 0–37 (16) |
| $D^1$ | 43 | 6–170 (49) | 553 | 0–54 (12) |
| $E^1$ | 32 | 0–133 (23) | 34 | 0–15 ( 6) |

The progeny of the various 4*n* crosses differed markedly in head production. For instance, the crosses 21 × 31 and 31 × 21 produced an average of 20 and 24 heads respectively in the $F_1$ generation, whereas 296 × 354 and 354 × 296 produced only 10 and 12 heads. Although there was much variation, Table VII shows that head productivity of the parental plants in their second

TABLE VII

HEAD PRODUCTION OF PARENTAL AND $F_1$ TETRAPLOIDS

| Parents (second year) | $F_1$ plants (first year) |
|---|---|
| More than 30 | 15.0 |
| 20 to 30 | 11.3 |
| Fewer than 20 | 10.7 |

year (when they were crossed) ran parallel with that of the progeny in their first year. Differences were less marked in the progeny, possibly because of the relatively sparse flowering of first-year plants.

*Florets*

The number of florets per head varied from plant to plant and with time of flowering, but was usually greater in tetraploids than diploids. The data in Table VIII were compiled from nearly 700 heads. The number of florets per head was least in the suppressed Series $A^1$ and greater in the luxuriant Series $B^1$. The general seasonal trend was toward higher numbers in the

TABLE VIII

RANGE AND AVERAGE NUMBER OF FLORETS PER HEAD

| Series | June | | July | |
|---|---|---|---|---|
| | $2n$ | $4n$ | $2n$ | $4n$ |
| $A^1$ | 47– 93 (70) | 57–105 (80) | 40– 78 (60) | 45– 99 (71) |
| $B^1$ | 40–109 (83) | 52–144 (100) | 56– 93 (75) | 61–114 (90) |
| $C^1$ | 40–106 (75) | — | 52– 98 (72) | 77–136 (97) |
| $E^1$ | — | — | 60–114 (85) | 68–144 (100) |

early heads and decreasing numbers in the later heads, but there were exceptions. On the whole the greater number of florets per $4n$ head was far outweighed by the superior number of diploid heads. In the floriferous, second-year plants of Series $B^1$ the total number of florets per $4n$ plant was only about 40% of that of diploids. The disparity was even greater in first-year plants (Series $D^1$ and $E^1$).

The linear dimensions of tetraploid florets averaged 25% greater than those of diploids. Structure was essentially similar but abnormalities were more frequent among tetraploids. For instance, in one plant the anthers were short and barely protruded beyond the cleft in the corolla tube. This individual was a poor pollen parent in controlled crossing. Other plants had florets with short, recurved rays allowing the style and staminal tube to thrust far out. A few plants with contorted, shrivelled florets interspersed through the heads were discovered to have cytological irregularities.

*Pollen*

Normal pollen grains were usually 28 to 30$\mu$ in diameter in diploids and 32 to 40$\mu$ in tetraploids. Grain size was more variable in the latter, some plants having consistently large (or less often relatively small) grains, while in others there were varying proportions of large and small grains, many defective. The pollen mother cells of the more variable plants generally revealed meiotic irregularities such as lagging chromosomes and bridges. A small minority of the tetraploids had consistently small, uniform, apparently normal grains. One plant with stomata above average size for tetraploids produced in one head grains of 35.5$\mu$ average diameter and in another grains of 31.5$\mu$, a size very little above that of diploids. Dermen (3) states that grain size is a more reliable criterion than size of stomata in the distinction of

diploids from tetraploids. This probably holds true for kok-saghyz, but in extreme cases there may be little difference in size.

Some plants, diploid as well as tetraploid, produced no pollen owing to abortion of the mother cells. Some such plants, especially among the diploids, were notably prolific head producers.

*Fertility*

Seed productivity tended to be lower in the $F_1$ tetraploids than in the diploids. Nearly a hundred heads of the optimal Series $B^1$ plants were bagged on three dates: June 5 to 7, when head production had shortly passed the maximum, June 19, and July 5, at the close of blossoming. The two heads of each cross were rubbed together a similar number of times and at seed collection the numbers of achenes and aborted ovaries were counted. Fertility in the diploids crossed on these dates was 53, 17, and 34% as compared with 22, 8, and 5% for tetraploids. Pollination may have been less effective than that by insects, and seed production correspondingly subnormal, but at least conditions were similar for $2n$ and $4n$ plants. Moreover, diploids and tetraploids both originated from random seed, so that the chances of relationship between crossed plants were remote.

About one hundred $F_1$ tetraploids of known parentage (Series $D^1$) were the subject of recorded crosses. Most sister plants proved intersterile, only 18% of the heads involved in 149 different crosses producing achenes. Fertility was no higher in 68 crosses with the reciprocal. When the $F_1$ plants had one parent in common, seed was set in 27% of the heads in 83 crosses. In 158 crosses between unrelated plants 54% of the heads produced achenes. Inadequate pollination was not the cause of failure; the heads were always thoroughly rubbed together and if any failed to open for any reason, such as cloudy weather, they were discarded.

A few tetraploids were exceptionally cross-fertile. For instance, one individual was fertile with four out of five sister plants. On the other hand, the cross-pollinated heads of the siblings were in each case completely barren, though at least two were fertile when crossed with unrelated plants. Tests of other heads revealed the fertile individual to be neither self-fertile nor apomictic. Evidently kok-saghyz has a complex incompatibility mechanism with occasional plants possessing a different genotype imparting unusual cross-fertility. Stout (11) has reviewed the literature on the inheritance of incompatibility.

## Roots

*Root Weight*

No outstanding differences in mean root weight were discovered between tetraploids and diploids. Table IX shows that tetraploids exceeded diploids in some series and were inferior in others. The biggest single root, weighing 79 gm., was tetraploid, but the distribution of size in tetraploid roots was little different from that in diploids. Ten per cent of all tetraploids (both first and second-year plants) possessed roots exceeding 30 gm., as compared with 12% of the diploids.

TABLE IX

RANGE AND MEAN FRESH-ROOT WEIGHT IN GRAMS

| Series | No. of plants | 2*n* | 4*n* |
|---|---|---|---|
| $A^1$ (singly in 6-in. pots) | 51 | 5.0–20.0 ( 8.9) | 4.0–16.0 (8.2) |
| $B^1$ (singly in 9-in. pots) | 31 | 7.0–31.5 (23.0) | 15.0–44.5 (26.0) |
| (two in 9-in. pots) | 23 | 7.0–25.0 (14.5) | 6.0–27.5 (16.5) |
| $C^1$ (singly in 8-in. pots) | 43 | 11.5–43.5 (25.0) | 6.7–33.0 (16.2) |
| (two in 9-in. pots) | 46 | 9.3–22.5 (16.6) | 6.2–24.5 (12.2) |
| $D^1$ (singly in 8-in. pots) | 513 | 7.0–32.5 (18.6) | 3.0–56.0 (17.8) |
| $E^1$ (singly in 8-in. pots) | 65 | 1.5–46.5 (20.1) | 6.0–79.0 (22.5) |

The smallest roots, both diploid and tetraploid, occurred in the suppressed Series $A^1$. The largest roots were developed by the second-year, single-potted plants of Series $B^1$, which began growth early in the greenhouse, became the leafiest and most floriferous, and were less affected by summer dormancy. In this series the tetraploids had slightly larger roots than the diploids. In Series $C^1$ where growth began later, outside, and summer dormancy was profound, the growth of the more lethargic tetraploids was seriously curtailed and at harvest the 4*n* roots were definitely smaller than those of the diploids. In the first-year plants (Series $D^1$ and $E^1$) that were started indoors in March, so that the 1945 growing period was longer than for Series $C^1$, the roots were of moderate size and without marked difference between tetraploids and diploids. Among the diploids, it is noteworthy that the progeny of colchicined plants selected as possible tetraploids but later proved diploid (Series $D^1$) were not superior but rather slightly inferior to the control plants derived from untreated field diploids (Series $E^1$).

Root weight in the controlled 4*n* crosses was variable and the data inconclusive. Results from the majority of the crosses studied are set forth in Table X. In some cases there was close agreement between both sets of $F_1$ progeny and the parents, as in 71 × 296 and 296 × 71. In other instances the two $F_1$ populations were similar but differed from the parents, as in the crosses 71 × 86 and 86 × 71. Yet another condition obtained in 17 × 31 and 31 × 17 when the progeny differed both from each other and the parents. When considering these results cognizance should be taken of the fact that the $F_1$ and parental generations had quite different histories, particularly in the early stages of development, and were grown under dissimilar conditions. Further, many of the parents were chimaeras with 4*n* rosettes and 2*n* roots. Hence the acquirement of adequate information requires the extension of selection experiments through the $F_2$ and later generations. It is noteworthy that in all crosses the sister plants exhibited less deviation from the mean root weight than the randomized control tetraploids in Series $E^1$. This probably indicates genetical influence. However, the nature of the mechanism is not apparent from the data at hand.

TABLE X

MEAN FRESH-ROOT WEIGHT OF PARENTAL PLANTS (SECOND YEAR) AND $F_1$ PLANTS (FIRST YEAR)

| Series | Description | Mean root weight of parents (gm.) | Root weight of $F_1$ plants | | Number of plants |
|---|---|---|---|---|---|
| | | | Mean | Standard deviation | |
| $D^1$ | $2n$ | — | 18.6 | — | 43 |
| | $4n$ All plants | — | 17.8 | — | 470 |
| | Cross 578 × 562 | 29.8 | 20.1 | 6.5 | 27 |
| | 31 × 21 | 26.5 | 16.7 | 6.9 | 26 |
| | 21 × 31 | 26.5 | 12.6 | 4.6 | 19 |
| | 31 × 17 | 26.5 | 21.0 | 6.3 | 33 |
| | 17 × 31 | 26.5 | 11.4 | 3.4 | 8 |
| | 31 × 257 | 22.0 | 24.1 | 11.5 | 17 |
| | 21 × 107 | 21.5 | 17.9 | 7.5 | 17 |
| | 17 × 296 | 21.3 | 15.3 | 6.2 | 10 |
| | 296 × 17 | 21.3 | 14.7 | 3.8 | 24 |
| | 31 × 86 | 20.0 | 24.3 | 11.8 | 38 |
| | 71 × 17 | 19.0 | 18.3 | 5.3 | 28 |
| | 296 × 354 | 18.3 | 14.4 | 5.0 | 27 |
| | 354 × 296 | 18.3 | 17.4 | 6.3 | 16 |
| | 466 × 469 | 17.5 | 9.9 | 3.4 | 10 |
| | 469 × 466 | 17.5 | 9.2 | — | 3 |
| | 71 × 296 | 16.3 | 15.0 | 5.0 | 17 |
| | 296 × 71 | 16.3 | 15.2 | 6.5 | 9 |
| | 621 × 619 | 15.7 | 15.4 | 3.8 | 17 |
| | 86 × 84 | 14.0 | 10.7 | — | 3 |
| | 71 × 86 | 12.5 | 20.1 | 10.5 | 16 |
| | 86 × 71 | 12.5 | 23.5 | 9.2 | 28 |
| | 70 × 86 | 8.5 | 23.2 | 8.2 | 14 |
| $E^1$ | $2n$ | — | 20.1 | 8.8 | 32 |
| | $4n$ | — | 22.5 | 15.3 | 33 |

*Root Habit*

The configuration of the root system of tetraploids tended to differ from that of diploids. This was demonstrated in Series $D^1$ and $E^1$ where both $2n$ and $4n$ plants were handled in the same manner, and damage to the roots was minimized by planting the germinating seedlings directly in small pots rather than in flats. There was much variation but stout undivided tap roots were more prevalent among the tetraploids. On the average the roots of tetraploids had 2.5 large, more or less equal divisions, the diploids 4.8 main parts.

## Relationships between Root Size and Other Characters

In order to determine whether correlation existed between the various vegetative and reproductive characters, data were assembled on root weight, head production, leafiness, and diameter of the rosette in the first-year diploids and tetraploids of Series $D^1$ and $E^1$, all of which were grown under similar conditions. The data are presented in Table XI.

TABLE XI

RELATIONSHIPS BETWEEN FRESH-ROOT WEIGHT, HEAD PRODUCTION, LEAFINESS, AND SIZE OF ROSETTE IN FIRST-YEAR DIPLOIDS AND TETRAPLOIDS

| Type | Root weight, gm. | No. of plants | Av. no. of heads | Av. number of leaves | | | Av. max. diam of rosette, in. |
|---|---|---|---|---|---|---|---|
| | | | | July 10 | At max. | Aug. 15 | |
| 2*n* | To 9.9 | 18 | 16.3 | 26.2 | 26.5 | 22.9 | 7.9 |
| | 10–19.9 | 29 | 36.0 | 41.8 | 48.2 | 43.0 | 9.3 |
| | 20–29.9 | 29 | 37.9 | 44.0 | 62.5 | 59.9 | 9.5 |
| | Over 30 | 7 | 53.3 | 46.7 | 70.0 | 70.0 | 10.4 |
| 4*n* | To 9.9 | 30 | 11.5 | 18.4 | 19.8 | 15.2 | 7.9 |
| | 10–19.9 | 141 | 12.8 | 20.3 | 26.1 | 25.1 | 8.8 |
| | 20–29.9 | 96 | 10.8 | 20.8 | 31.1 | 30.8 | 9.4 |
| | 30–39.9 | 37 | 9.9 | 23.3 | 45.0 | 44.0 | 10.0 |
| | Over 40 | 10 | 10.1 | 26.7 | 54.1 | 54.0 | 11.6 |

Absence of flowering during the first year has been reported in the Russian literature, reviewed by Krotkov (7), as associated with large root size in diploid kok-saghyz, and it was suggested that selection should be made on that premise. The data in Table XI clearly do not support such a conclusion. Among the diploids head production during the first year was extremely variable, as illustrated by the range, 7 to 170 heads in the largest specimens (with roots exceeding 30 gm.), but, on the whole, plants with the biggest roots were the most floriferous, and conversely those with the smallest roots tended to have fewest heads. In the tetraploids, where the number of plants was greater, a different trend appeared. Here flowering was much reduced, with the maximum in plants possessing roots somewhat below average size (10 to 19.9 gm.). However, because of the great variability among individuals it was clear that neither high nor low productivity of heads, in either diploids or tetraploids, could be considered prognostic of ultimate root weight. There were indications that the smaller plants tended to flower earlier than those that finally became largest, but dates of the initiation and duration of flowering were not recorded.

Leaf shape was another character regarded by one Russian investigator as useful for selecting kok-saghyz, but has been discounted by others (Krotkov (7) ). Records were not kept of the leaf shape of all the first-year tetraploids (Series $D^1$ and $E^1$) but the available data are given in Table XII. At least in

TABLE XII

FRESH-ROOT WEIGHT AND LEAF TYPE IN FIRST-YEAR TETRAPLOIDS

| Root weight (gm.) | Distribution of plants according to leaf type | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| To 9.9 | 1 | 13 | 22 (45%) | 13 | 0 |
| 10–19.9 | 3 | 61 | 90 (39%) | 78 | 1 |
| 20–29.9 | 0 | 23 | 40 (44%) | 28 | 0 |
| Over 30 | 0 | 7 | 11 (41%) | 6 | 3 |

these plants there was no obvious relationship between leaf type and root weight.

Nichiporovich and Ivanitzkaja (9) reported a correlation between numbers of leaves produced during the period of most active growth and ultimate root size in diploid kok-saghyz. A generally similar relationship was observed in the first-year plants, both diploid and tetraploid, of Series $D^1$ and $E^1$ (Table XI). Although highly variable, plants that were leafiest in July tended to produce the biggest rosettes and roots. However, it is clear from the data that the larger size of these plants was due not so much to more rapid early growth as to lengthening of the growing period. The rosettes of plants that produced the smallest roots, weighing up to 9.9 gm., in most cases had achieved full expansion in early July and by Aug. 15 had undergone considerable diminution. While the rosettes of the small plants were dying back, those of the large plants continued to expand, and in the case of the biggest this expansion was not completed until mid-August (Table XI). The relative state of leafiness on Aug. 15 as contrasted to July 10 was in the order of $-13$, $+13$, $+36$, and $+50\%$ in the four categories of diploids with progressively larger roots, and $-16$, $+24$, $+48$, $+89$, and $+102\%$ in tetraploids. The greater increase during the latter part of the growing season in tetraploids is further illustration of their physiological tardiness. They grew more slowly than diploids but duration of the first cycle of growth extended deeper into the summer.

Of the various characters studied, diameter and leafiness of the rosette appeared to be the best indicators of the ultimate root size. However, since the average differences during the early stages tended to be slight, especially in the tetraploids, and the variation among individuals was great, this character proved of limited value when put to actual test. Many of the plants selected for crossing because of their promising appearance at time of flowering were finally found to have roots of average size. Conversely the potentialities of other plants were unrecognized in early summer because much of their ultimate bulk was due to prolonged rather than rapid growth. The attempts at selection for root size during early summer were generally discouraging, and it seemed clear that a longer program was required with crossing deferred until after the completion of summer growth.

## Discussion

In the present paper comparative data are presented on three categories of kok-saghyz plants grown in pots. The types are random diploids, $F_1$ diploids derived from colchicined plants selected as possible tetraploids on their leaf characters but later determined as predominantly diploid, and the $F_1$ generation of colchicine-induced tetraploids.

The progeny of selected colchicined diploids differed from the random derivatives of untreated field diploids in possessing leaves that were slightly wider, less deeply dissected, and somewhat less numerous. In these leaf characters they resembled the parental plants. Size of stomata, however, was much reduced and approximated that in random diploids. Rosettes were no larger, and root size was slightly below that of random diploids, though the inferiority was so minor as probably to be without significance.

The $F_1$ tetraploids exhibited the morphological modifications described repeatedly in the extensive literature of polyploidy. All parts of the plant body were more robust than in diploids. The leaves were usually wider and less dissected, but the increase in width was counteracted by the marked reduction in number, and only at maturity did leaf area of the tetraploids approximate that of the diploids. Similarly $4n$ inflorescences were larger and contained more florets, but the heads were so reduced in number that even second-year tetraploids produced only 40% as many florets as diploids. Since fertility was also definitely lower, achene production was but a small fraction of that in $2n$ plants.

Sibling tetraploids were notably intersterile, fewer than one-fifth of the crosses between sister plants being successful. With decrease in genetic relationship cross-fertility increased. As a rule the induction of tetraploidy brought no release from the operation of the incompatibility mechanism possessed by kok-saghyz. Reports on other genera indicate varied effects of polyploidy on incompatibility. Stout (12) described the origin of self-fertile $4n$ petunias from self-sterile $2n$ plants. Lewis and Modlibowska (8) found a definite increase in self-fertility in tetraploids derived from an only slightly self-fertile diploid clone of the cultivated pear. On the other hand, Hecht (5) reported persistence of self-sterility in tetraploid *Oenothera rhombipetala*. Evidently kok-saghyz belongs in that category of plants whose incompatibility mechanisms persist despite polyploidy.

No outstanding differences in mean fresh-root weight were discovered between tetraploids and diploids, the former showing minor superiority in some series and slight inferiority in others. The roots of the tetraploids were usually more robust, but the increase in thickness was offset by reduction in number of major divisions so that the average weight was not greatly different.

In their growth behaviour tetraploids were more torpid than diploids. Individuals were highly variable, but generally the rosettes of tetraploids underwent slightly slower increase in diameter, leaf turnover was greatly reduced, flowering began slightly later, head production was much lower,

fertility greatly reduced, and summer dormancy tended to be postponed. This tardiness seems characteristic of tetraploids generally. Causal factors have not been investigated in many cases, but Noggle (10) in his report on rye described the tetraploids as being physiologically younger than diploids of similar chronological age. Avery and Pottorf (1) and Gustafson (4) found the concentration of growth hormones to be lower in tetraploids.

In the various $F_1$ generations of tetraploids produced by controlled crossing, sister plants exhibited less deviation from the mean than randomized plants in size, shape, and number of leaves, production of heads, and size of roots. Of these characters, leaf shape provided the clearest demonstration of an inheritance mechanism. Head production also appeared to be inherited though not so precisely as leaf shape. The data on root weight were less conclusive and selection experiments need to be continued to the $F_2$ and succeeding generations.

It should be emphasized that the descriptions in this paper refer to potted plants. There were indications both from these and from plants grown in the field that when the more lethargic tetraploids were given opportunity to achieve full growth, they sometimes surpassed diploids. Warmke (13) reported the roots of field-grown tetraploids to be twice as heavy as those of diploids, but the number of plants was small. In plots at Toronto the tetraploids exceeded diploids by a small but definite margin. In general the tetraploids gave the impression of having possibilities of surpassing diploids, but seemed held back by undetermined deficiencies in their growth mechanism. Whether the growth characteristics of the outstandingly large tetraploids are heritable remains to be discovered.

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## References

1. Avery, G. S., Jr. and Pottorf, L. Polyploidy, auxin and nitrogen in green plant tissue. Am. J. Botany, 32 : 669-671. 1945.
2. Bannan, M. W. Tetraploid *Taraxacum kok-saghyz*. I. Characters of the leaves and inflorescences in the parental colchicine-induced generation. Can. J. Research, C, 23 : 131-143. 1945.
3. Dermen, H. Colchicine polyploidy and technique. Botan. Rev. 6 : 599-635. 1940.
4. Gustafson, F. G. Growth hormone studies of some diploid and autotetraploid plants. J. Heredity, 35 : 269-272. 1944.
5. Hecht, A. Induced tetraploids of a self sterile Oenothera. Genetics, 29 : 69-74. 1944.
6. Kern, E. M. Right-angle grid system for mapping plant distribution. Ann. Missouri Botan. Garden, 32: 283-286. 1945.
7. Krotkov, G. A review of literature on *Taraxacum kok-saghyz* Rod. Botan. Rev. 11: 417-461. 1945.

8. LEWIS, D. and MODLIBOWSKA, I. Genetical studies in pears. IV. Pollen-tube growth and incompatibility. J. Genetics, 43: 211-222. 1942.
9. NICHIPOROVICH, A. A. and IVANITZKAJA, E. F. On the relation between leaf development and formation of laticiferous vessels in roots of kok-saghyz and krym-saghyz. Compt. rend. acad. sci. U.R.S.S. 44: 33-36. 1944.
10. NOGGLE, G. R. The physiology of polyploidy in plants. Thesis abstract. Urbana, Ill. 1945.
11. STOUT, A. B. The genetics of incompatibilities in homomorphic flowering plants. Botan. Rev. 4: 275-369. 1938.
12. STOUT, A. B. Inactivation of incompatibilities in tetraploid progenies of *Petunia axillaris*. Torreya, 44: 45-51. 1944.
13. WARMKE, H. E. Experimental polyploidy and rubber content in *Taraxacum kok-saghyz*. Botan. Gaz. 106: 316-324. 1945.

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